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THE SIGNIFICANCE OF AURICULAR FIBRILLATION AND AURICULAR THROMBOSIS IN MITRAL VALVE SURGERY

DWIGHT C. McGOON AND WALTER S. HENLY

Department of Surgery, The Johns Hopkins Hospital and The Johns Hopkins University School of Medicine

Received for publication August 26, 1952

That auricular fibrillation is of common occurrence in rheumatic valvular heart disease has been realized for many years. That thrombus formation occurs in much greater frequency in auricles which are fibrillating is also apparent from past experience. This and related knowledge have been of value in the formulation of the prognosis of the disease, and in the consideration of the use of quinidine in the treatment of auricular fibrillation.

Fresh interest in this subject is stimulated by the relatively recent successes in the amelioration of valvular lesions in rheumatic heart disease by surgical means. It seems obvious that such manipulations upon a heart so severely diseased as to exhibit auricular fibrillation might prove unduly dangerous, or that procedures within an auricular chamber containing thrombus could loosen portions of the thrombus which would then acquire the role of extremely dangerous emboli. Because of these factors, statistical information regarding the incidence of auricular fibrillation and auricular thrombus formation in such patients, and the surgical hazards thus incurred, is of interest to the cardiac surgeon.

The sources of accurate information on incidence, surgical prognostic importance, and related statistical data of auricular fibrillation and auricular thrombus formation in valvular heart disease are limited. Detailed autopsy files combined with complete clinical records are one valuable source of such information, but bear the handicap of representing cases in the end stages of the disease only. The other source of data, namely operative experience, is of more pertinence as regards the type of material, but lacks completeness in that examination of the heart cannot be thorough. Since these two sources represent the limit of available knowledge on this subject, the autopsy files and operative experience at this hospital, and similar studies recorded in the literature, have been analyzed.

AUTOPSY SERIES

During the fifteen years following 1935, complete and detailed clinical and autopsy records are available on 89 cases of mitral stenosis. The series was not otherwise selected, the mitral stenosis often being associated with other valvular lesions, and proof of rheumatic origin was not considered necessary for

inclusion in the series. However, 15 cases of subacute bacterial endocarditis associated with mitral stenosis were excluded, since this complication is rarely encountered in the chronic type of valvular lesion, and also since such patients are excluded from surgical treatment. Patients in each decade of life through 70 years were encountered. The representation of each sex was equal.

Auricular Fibrillation and Thrombosis

Of the entire group of 89 cases, 50% were found to have cardiac arrhythmias (hereafter called auricular fibrillation, since this is the overwhelmingly predominant arrythmia; there were only two exceptions, one patient having paroxysmal auricular tachycardia with left auricular thrombosis, and one patient having nodal rhythm without auricular thrombosis). Of the entire group of 89, 30% of the hearts were found to contain auricular thrombi, as follows:

In the left auricle alone	12 cases
In the right auricle alone	6 cases
In both auricles	9 cases
Total	27 cases = 30%

In the 50% of the patients who had had fibrillation during life, 56% were found to contain thrombus material in the auricles at autopsy. However, in the 50% of the patients who had not fibrillated, only 5% contained thrombus material in the auricles. Thus there is a very significant correlation in the incidence of auricular thrombosis in patients with and without auricular fibrillation.

Similar figures may be stated in a converse manner. Of the 27 patients from the entire group exhibiting auricular thrombosis, 93% had had auricular fibrillation. Thus it is almost constant to find fibrillation during life in those patients exhibiting auricular thrombosis at autopsy. The occurrence of fibrillation, however, does not necessarily imply that auricular thrombosis has occurred, for of the 62 patients from the entire group who showed no auricular thrombosis at autopsy, 20 (31%) also had had auricular fibrillation.

The relationship between the duration of fibrillation and the incidence of auricular thrombus formation could not be obtained from this study, due to the frequent lack of information concerning the date of onset of the fibrillation.

The average duration of life following the onset of severe symptomatic heart disease was four years in the group exhibiting auricular thrombosis, as opposed to only 2.2 years in those without thrombi. It would thus seem that the more chronic cases tend to have auricular thrombosis more frequently. The average ages at death in the group with and without auricular thrombus formation were nearly identical, being 40.8 and 38 years respectively. The average ages at death of the groups having had and having not had fibrillation were 41.5

and 37 years respectively, or essentially similar. Thus age of the patient appears of negligible importance in this study.

Systemic Embolism

The presence of thrombus material in the left auricle would be expected to result in the breaking off of emboli into the systemic circulation in a high percentage of cases. Of the 21 instances in which left auricular thrombosis was found in this series, clinical evidence of systemic embolism, confirmed at autopsy, was present in 11, or 52%. In the 68 cases not having left auricular thrombosis, infarcts suggesting embolism were found in only five patients, and even in these it was interesting to note that *in each* auricular fibrillation had been present during life. (The kidney, spleen, and brain were the most commonly involved organs in both groups.) So that in the entire group of cases exhibiting evidence of systemic embolism, auricular fibrillation had been present in all. Thus, in this series, auricular fibrillation was the universal precursor of spontaneous systemic embolism.

Miscellaneous

When thrombus was found in the left auricle, the appendage was almost uniformly involved, either alone or in combination with involvement of the atrium proper. In only 14.3% did the atrial wall have thrombus attached without thrombus also in the appendage, and in 52% thrombus was present in the appendage only, without attachment to the atrial wall. This is in close agreement to similar studies of Jordan, Scheifler, and Edwards (1).

Heart weight was evaluated by determining its percentage of total body weight. The average percentage in all 89 cases was 0.87%, in all cases without auricular thrombus formation 0.83%, in all cases with auricular thrombosis 1.0%, and in all cases with auricular fibrillation 0.96%. In general, therefore, fibrillation and auricular thrombus formation were associated with greater cardiac hypertrophy.

Occasionally at operation the pericardial cavity will be found to be obliterated by adhesions. Such binding down of the appendages might be feared to predispose to thrombus formation. However, such adhesions were present in only 3.7% of the cases with auricular thrombosis, and in 9.7% of the remainder. Thus such predisposition of pericardial adhesions to thrombus formation seems unsubstantiated.

There was very little difference in the activity of the rheumatic lesions in the hearts, as determined by microscopic study, whether associated with auricular thrombosis or not, and little difference in the incidence of occurrence of a "MacCallum's patch" in the two groups.

Literature

The results of this study are compared in Table I with the statistics from five similar investigations (2, 3, 4, 5, and 6). The averages of these recorded statistics, including those presented in this article, are also calculated. The figures fall into consistent patterns in the various reports, and into well correlating ranges of magnitude. The data of other investigators are also in agreement (7 and 8).

OPERATIVE SERIES

This series consists of the first 58 patients who have been operated upon for mitral stenosis by Dr. Alfred Blalock and his associates at this hospital, with a period of from 32 to 3 months having elapsed since the operative procedure. These patients were selected for operation according to the criteria of Andrus (9). The valve was approached through the left auricular appendage, a portion of which was submitted for pathological study. The presence of thrombus in the appendage could thus be detected by two means: by palpation and visualization at operation, and by gross and microscopic pathological examination.

Auricular Fibrillation and Thrombosis

Of the group of 58 patients, 22 (38%) were fibrillating prior to operation. (It should be mentioned that the appearance of fibrillation during the immediate postoperative course occasionally occurred, but this was almost uniformly transient, and at no time was associated with evidence of embolism.) Of the entire group of 58 patients, 12 (21%) showed evidence of left appendage thrombosis. Of the 38% of patients having auricular fibrillation, 10 (45%) showed evidence of thrombus in the left appendage, whereas in the 62% of non-fibrillating patients, only 2 (5.5%) of the appendages contained thrombus.

Of the 12 patients showing evidence of appendage thrombosis on pathological examination of the appendage biopsy (7 of whom were also noted to have thrombus material in the appendage at the time of operation), 10 (83%) had exhibited fibrillation, and 2 (17%) had not.

Systemic Embolism

Of the entire group of 58 patients, four (6.9%) showed evidence of embolism which occurred sometime during the period of operative anesthesia. In each of these, cerebral embolism took place, as manifested by hemiplegia and other neurological signs of cerebral injury. In addition to the cerebral embolism, in one patient lodgement of emboli in the iliac and femoral arteries occurred, these being successfully removed immediately.

Fortunately, death occurred in only one case of cerebral embolism, but another patient has remained aphasic and hemiplegic, and in this patient elec-

TABLE I

Accumulated Statistics on the Relationship Between Auricular Fibrillation and Auricular Thrombosis in Mitral Stenosis

INVESTIGATORS	INCIDENCE OF FIBRILLATION	INCIDENCE OF MURAL THROMBOSIS	INCIDENCE OF MURAL THROMBOSIS IN CASES WITH FIBRILLATION	INCIDENCE OF MURAL THROMBOSIS IN CASES WITH REGULAR RHYTHM	INCIDENCE OF FIBRILLATION IN CASES WITH MURAL THROMBOSIS	NUMBER OF CASES IN SERIES	TYPE OF STUDY
	%	%	%	%	%		
Hay and Levine	57	34	49	14	83	186	Mitral stenosis at autopsy (S.B.E. excluded)
Garvin	55	30	43	18	74	116	Adult autopsies with rheumatic heart disease
Graef et al.	—	13	—	—	63	178	Autopsied hearts with rheumatic heart disease
Weiss and Davis	57	19	30	5	88	131	Autopsied hearts with r. h. d. the cause of death
Stone and Feill	53	37	44	33	65	100	Advanced mitral stenosis at autopsy
Johns Hopkins	50	30	56	4	93	89	Mitral stenosis at autopsy (S.B.E. excluded)
Average (autopsy group)	55	26	44	15	78	133	
Johns Hopkins (operative series)	38	21	45	5.5	83	58	Cases operated upon for mitral stenosis

troencephalographic and angiographic evidence indicate complete occlusion of the left anterior and middle cerebral arteries. A third patient remains hemiplegic but active, and in the fourth the signs of hemiplegia have nearly completely disappeared.

Three of these patients who developed cerebral embolism had pre-operative auricular fibrillation, or an incidence of embolism of 13.5% in the fibrillation group. Thus only one patient who had not fibrillated developed embolism, or only 2.8% of the non-fibrillating group. Likewise, of the 12 patients with auricular thrombosis, three developed postoperative embolism (25%), whereas only one such embolism occurred in the 46 patients without auricular thrombus formation (2.2%).

Relationship to Mortality

No deaths occurred at the time of surgery. Five deaths (8.6%) occurred in the postoperative period, i.e. before discharge from the hospital. One of these

TABLE II

Summary of Data on the Operative Series of Mitral Commissurotomy Patients

	PER CENT OF PATIENTS			
	with	*without*	*with*	*without*
	Auricular fibrillation in the groups below		Auricular thrombosis in the groups below	
Entire group	38	62	21	79
Fibrillation	—	—	83	17
Auricular thrombosis	45	5.5	—	—
Systemic embolus	13.5	2.8	25	2.2
Death	18	0	25	2.2

died 10 weeks postoperatively of an extraneous operative complication, namely purulent pericarditis and innominate vein thrombosis. The remaining four deaths occurred within the first three days after operation, and are considered to be caused directly by the operative procedure. Of these four, all had had auricular fibrillation. The immediate mortality in the group with fibrillation was thus four of 22 (18%), and in the group without fibrillation, 0%. This is compared to the over-all immediate postoperative mortality of 6.9%.

Of the 12 patients showing auricular thrombosis, three experienced postoperative death (25%). One died of cerebral embolism (autopsy), one died on the third day following operation of "exhaustion" (no autopsy), and one on the day following operation with hyperpyrexia (no evidence of embolism at autopsy). In the remaining 46 patients in whom no thrombus was present, only one postoperative death occurred, or 2.2%. Actually in this one patient calcification in the wall of the left auricle at autopsy was interpreted as "possibly a partially calcified thrombus." These data are summarized in Table II.

DISCUSSION

The figures recorded in Table I indicate that the incidence of mural thrombosis, and of fibrillation, and of the inter-relationship of these two factors, are similar in the autopsy and operative data. The fact that auricular fibrillation and thrombosis are less frequent in the operative series is consistent with the fact that operative intervention occurs at an earlier stage of the disease than does death.

However, in the series of Harken (10), 60% of the patients submitted to surgery had auricular fibrillation. Eight of his patients developed peripheral emboli, with fibrillation present in 75% of these, or exactly the same percentage as in the series recorded here.

Bailey and his group (11) found that in their 235 cases, 12 (5.1%) had developed cerebral embolism. Because of this incidence, they have utilized a technique of carotid artery occlusion during intracardiac manipulations which has eliminated this complication in the 80 patients on whom it has been tried. Although in Bailey's series the incidence of auricular fibrillation in the patients developing cerebral embolism is not stated, the data of the Johns Hopkins series indicate that the risk of carotid occlusion may prove justifiable only in patients having auricular fibrillation.

In the operative series of this report, the great majority of unfortunate results befell those patients having preoperative auricular fibrillation. All four deaths directly resulting from the operative procedure, and 3 of the 4 peripheral embolic phenomena, occurred in this group of patients. It should not necessarily follow, however, that auricular fibrillation is a contraindication to operation. Such patients are usually more severely ill, and in more desperate need of operative intervention. The knowledge of this increased operative risk should merely fit as a link in the chain of evidence for and against the decision of operative intervention.

SUMMARY

1) In the autopsy series, auricular fibrillation occurred in 50% of patients with mitral stenosis during the course of the disease, and auricular fibrillation occurred in 38% of patients before operative intervention was performed (mitral commissurotomy).

2) Of the patients with fibrillation, approximately half were found to have auricular thrombosis.

3) The great majority of patients with auricular thrombosis had had auricular fibrillation.

4) Of the patients who fibrillated prior to operation, 13.5% developed cerebral embolism during the period of anesthesia, but only 2.8% in the non-fibrillating group experienced such embolism.

5) Of the patients in the operative series with auricular thrombosis, 25% developed cerebral embolism, whereas in patients without auricular thrombosis, such embolism occurred in only 2.2%.

6) It is thus apparent that auricular fibrillation is a definite operative hazard, as is further evidenced by the pre-operative presence of auricular fibrillation in each of the four patients in this series dying as a direct result of the operative procedure.

7) This does not imply that patients with mitral stenosis associated with auricular fibrillation should be denied mitral commissurotomy.

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COMPARATIVE SUSCEPTIBILITY OF CULTURED CELL STRAINS TO THE VIRUS OF EASTERN EQUINE ENCEPHALOMYELITIS

FREDERIK B. BANG AND GEORGE O. GEY
With the technical assistance of M. FOARD AND D. MINNEGAN
Departments of Medicine and Surgery, The Johns Hopkins University School of Medicine

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Many viruses have now been grown by various methods of tissue culture (1), but in only a few instances under circumstances which allowed an adequate analysis of the conditions of growth of both virus and cell and a study of the types of cells infected (2). Wide variation in the destructive effect of a virus (eastern equine encephalomyelitis) on strains of rat cells when these are grown in continuous roller tube cultures (3, 4) is here reported. This selectivity occurs in the same medium with a maximum number of cells exposed to virus. The greatest difference in susceptibility was between one normal strain which was resistant as compared to its tumor cell derivative, which was rapidly destroyed. Other cell strains were of intermediate susceptibility and produced a low yield of virus for six to nine months after inoculation. Cell growth continued despite the presence of virus.

MATERIALS AND METHODS

Virus

The *eastern strain of equine encephalomyelitis* was obtained originally from Dr. Carl TenBroeck (5) and has been used by us in previous tissue culture studies (6). This virus continues to have a high degree of neurotropism when passed in chick embryos as demonstrated by the production of encephalitis following the inoculation of guinea pigs in the foot pad. Tubes of 10 per cent infected embryo suspensions were kept frozen in celloidin screw cap tubes refrigerated with solid CO_2. The virus maintained its titer for several months. It was inoculated into the cultures immediately after dilution in cold physiological saline (0.85% NaCl) as indicated in the individual experiments. Titrations of the amount of virus in the supernatant fluid from the cultures were carried out by inoculation of serial ten fold dilutions on 10 or 11 day old chick embryos (7) and the 50 per cent mortality within three days calculated (8). This has given a variation of .6 log on replicate titrations (7), which is about twice the standard error when using five embryos in each dilution (9). Occasionally when surveying different cell lines for susceptibility to this virus the titrations were limited to as few as two embryos inoculated with each dilution. These are indicated in the text. This raises the standard error of the LD_{50} to .6 log (9). All material obtained from the tissue cultures was either titered immediately or frozen at −16°C. and titered within the next week. Repeat titrations on frozen material within this time showed no more variation than indicated by the error of the titration. When only one or two em-

Supported in part by Grants-in-Aid from the U. S. P. H. Service, Federal Security Agency and the American Cancer Society.

bryos in the first dilution died from such inoculation, this embryo was further tested by subinoculation on two embryos. If both of these embryos died in 36 hours without bacteria, the original death was considered as a virus death. Charts representing the amount of virus recovered from each tube are presented with the protocols. When undiluted tissue culture fluid was inoculated into eggs and only one of the number of embryos used died, then this calculated 50 per cent end point fell below 10^{0}. We have given an arbitrary value to such results by calculating a point between 10^{0} and 10^{+1} (10 drops of fluid on one embryo) which is the point at which half of the embryos would be expected to die. This was done only when the isolated embryo deaths were proven to be caused by virus.

Rat Cell Strains

*Autologous Rat Strains**—Five of the strains studied were derived in culture from tissue explanted from the same rat in 1938. Four of these originated from the same subcutaneous areolar tissue explant (10). The normal cell strain, *14p*, produced the tumor strain *T-333* during continuous cultures as previously described (4). The malignant cell strains *TSAT-72-624* and *TSAT-2936* were produced by the inoculation of an altered strain, *ANSAT-21* into rats. This altered strain arose in tissue culture from the same explants as *14p*. The strain *ARF* was derived from the articular cartilage of the femur of the same rat which supplied the subcutaneous areolar tissue strain *14p*. Mrs. Margaret K. Gey kindly supplied the starting stocks of these strains.

*Homologous Rat Strains**—Four *M* strains of normal fibroblasts were derived from the muscle of four young adult rats. All cell strains had been in continuous culture about two years when studied. Three *Sk* strains of normal fibroblasts were derived from the dermis of three young adult rats and were of the same culture age. We are greatly indebted to Mrs. Mary Toye Kubicek for supplying this group of cultures.

Walker Rat Carcinoma 256 (11)—This strain had been maintained in tissue culture by Mrs. Gey for about eight years.

Human Cell Strains

Strain *D-1 Re* was derived from a subline of the chondromyxosarcoma strain *J Da* explanted in 1931 (3). Strain *A Fi* was derived from a fibrosarcoma explanted in 1935. Mrs. Gey kindly supplied both strains. During the greater period of their cultivation they have continued to grow as stable strains.

Culture Medium

The medium used for the cultivation of the cells varied somewhat from experiment to experiment and is described in the individual protocols. In this paper the culture medium formula 1-1-4-4 refers to a clotted substrate formula of 10 per cent balanced salt solution (Gey), 10 per cent bovine embryo extract, 40 per cent human placental cord serum and 40 per cent chicken plasma. The formula 4-1-5-0 refers to a liquid medium containing 40 per cent balanced salt solution, 10 per cent bovine embryo extract and 50 per cent human placental cord serum. All experiments here reported, unless otherwise specified, were done with continuous cultures maintained in 16 x 150 mm Pyrex roller-tubes rotating at 12 revolutions per hour and incubated at 37°C. in the 4-1-5-0 fluid medium. In this formula the last figure (-0) indicates the absence of plasma, thus no clot can form.

* In this paper "autologous" refers to strains of cells derived from one individual rat and "homologous" as derived from the same species.

Electron Microscopy

During the early part of these studies, drilled or punched slide cultures (3) were prepared for electron microscopy by a slight modification of Porter's procedure (12). In this modification two formvar coated coverslips (40 x 45 mm) were sealed on opposite sides of a 2 x 3 inch stainless steel slide with a 35 mm hole. The cells were grown on the formvar coating in a medium of 25 per cent cord serum in balanced salt solution. The medium made contact with both coverslips, and the cells were therefore maintained in a partially anaerobic environment. Fixation with osmic acid vapor was limited to 10 to 15 minutes at 37°C. To avoid spread of oily materials over the preparation during rinsing, the use of vaseline seals was eliminated. A high paraffin content seal was used only on the outside. This was removed as completely as possible before washing with normal physiological saline (0.85% NaCl) at 37°C for a few minutes. After fixation, the preparations were further washed with saline and then water or with water alone. Parts of the wet membrane with cells were harvested under the binocular microscope and immediately placed on the electron microscope screen and dried.

Subsequently, when it became important to harvest cells directly from the roller tube cultures, certain modifications were introduced. Roller tubes and other continuous culture chambers offer distinct advantages over coverslip slide preparations by maintaining more favorable physiological conditions for cell cultivation over long periods. In order to obtain electron microscope visualization of such cells, a method of coating the inside of these tubes was developed. This avoids the use of the roller tube slip (3). The method used for applying the formvar plastic coating produced membranes which were not loosened by the washing action of the culture media.

New, previously unused Pyrex roller tubes were cleaned with Dupanol C*, washed with distilled water rinsed in 95% alcohol and dried. The membranous coating was produced by filling the tube with a 0.25 per cent solution of formvar in ethylene dichloride. When this was decanted, it left a thin uniform film on the tube wall. This was dried by aspiration with a water pump. The coated tubes could be stored on a sterile rack in a moist chamber and were satisfactory for as long as a month. The cells in such coated tubes were found to be more thinly spread out than those on the static slide preparations. Washing and fixation of the cultured cells was similar to the slide procedures. The cell coated membrane could be removed by breaking open the tubes and removing the membrane from the fragments.

PROTOCOLS

A Comparison of Susceptibility of a Tumor Cell Strain (T-333) with Its Normal Prototype (14p)

The purpose of this first group of experiments (Charts 1 and 2) was to compare the susceptibility of a normal strain with an autologous malignant strain previously derived from it during continuous culture. The first two experiments were performed in clotted media, the last two in liquid media.

Experiment I (Chart 1)—A roller tube culture of an eleven-year-old normal rat fibroblast strain, *14p*, was selected. This culture had been grown in the composite medium 1-1-4-4 containing 10 per cent B. S. S.; 10 per cent B. E. E., 40 per cent human placental cord serum, and 40 per cent chicken plasma as clotted substrate. The supernatant culture

* Obtained from E. I. DuPont de Nemours Co., Wilmington, Delaware.

fluid was 40 per cent B. S. S. (Gey), 10 per cent B. E. E. (bovine embryo extract), and 40 per cent Cd. S. (human placental cord serum). Individual roller tube cultures were started as follows: three drops of the fluid components 1-1-4 and two drops of the chicken plasma were added to the tube (16 x 150 mm. Pyrex), mixed thoroughly together with a curved-tip tissue pipette (Gey), and spread throughout the lower two-thirds of the roller-tube as a thin film. Four fragments of the cultured strain were immediately added, thor-

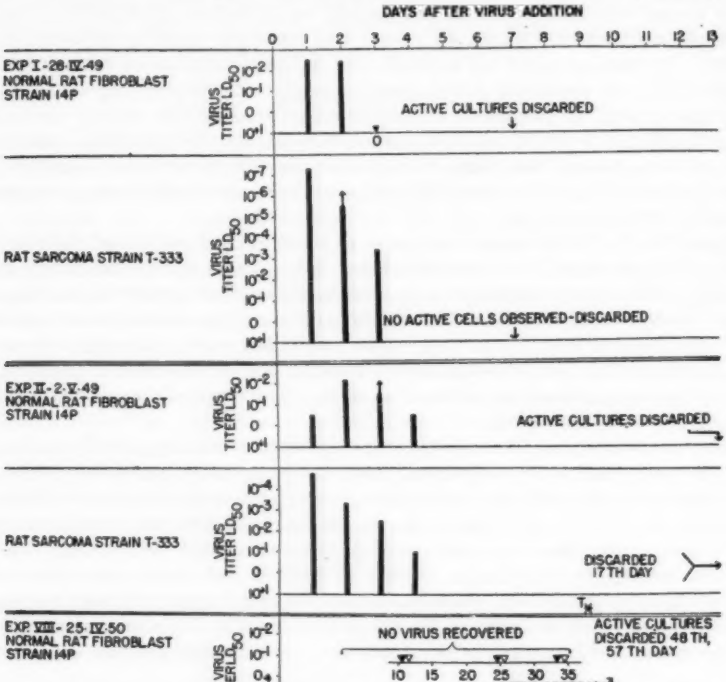

DAYS AFTER VIRUS ADDITION
0 1 2 3 4 5 6 7 8 9 10 11 12 13
EXP I - 28-IX-49
NORMAL RAT FIBROBLAST
STRAIN 14P
VIRUS TITER LD50
ACTIVE CULTURES DISCARDED
RAT SARCOMA STRAIN T-333
NO ACTIVE CELLS OBSERVED-DISCARDED
EXP II - 2-X-49
NORMAL RAT FIBROBLAST
STRAIN 14P
ACTIVE CULTURES DISCARDED
RAT SARCOMA STRAIN T-333
DISCARDED 17TH DAY
EXP VIII - 25-IX-50
NORMAL RAT FIBROBLAST
STRAIN 14P
NO VIRUS RECOVERED
ACTIVE CULTURES DISCARDED 48TH, 57TH DAY
10 15 20 25 30 35
VIRUS ADDED 10-3
RAT SARCOMA STRAIN T-333
COLONIES DESTROYED – VERY FEW ACTIVE CELLS - DISCARDED

CHART 1. A comparison of the virus yield of susceptible tumor cell (T-333) with the yield from the resistant normal cell (14p). Three experiments. *T = transfer. Inverted triangle designates negative virus assays.

oughly wet with the composite medium, and oriented in line in the bottom half of the tube. Three drops of the mixed fluid were discarded before clotting took place, and the remainder was allowed to clot over the partially embedded tissue cultured fragments. Ten drops of the supernatant fluid phase 4-1-5-0 composed of 40 per cent B. S. S., 10 per cent B. E. E., and 50 per cent Cd. S. were then added. After the cells had grown four days, one drop of a 10^{-4} dilution of EEE virus was added.

The rat sarcoma strain *T-333* arose from the normal strain *14p* during continuous culture when the latter was over two years old. These malignant cells were set up in roller tubes at the same time and in the same medium as used for the normal cells. One drop of a 10^{-4} dilution of the same virus suspension was added to the newly transferred cultures.

The supernatant fluids from the sarcoma culture on the first day showed 100,000 times as much virus as was present in the normal cell lines, and by the seventh day only cell debris and dead cells were seen in the sarcoma cultures. The normal cultures were at this time healthy and, on direct observation, showed many dividing cells.

Experiment II—A roller tube culture of the normal strain *14p* was set up in the same manner as in Experiment I, but in this instance the cultured fragments selected were cut up at the time of the transfer in a 10^{-3} suspension of virus. Despite this intimate exposure, these cultures remained in good condition and were transferred nine days later, and then, while still healthy, were discarded on the thirteenth day.

The *T-333* sarcoma strain was transferred into another portion of the medium which was used for the normal prototype strain *14p* and was similarly treated with virus. Transfer (T, chart 1) the damaged culture was attempted nine days following virus addition, and the culture carried until the seventeenth day, but then was discarded. Again the supernatant fluid from the malignant cells yielded more virus.

Viral Production in Fluid Media

In order to avoid the effects of the barrier to virus penetration produced by a fibrin clot (2), viral production was studied in roller tube cultures maintained in fluid media only.

Experiment VIII (Chart 1)—This was part of a larger experiment in which the viral susceptibility of a number of unrelated cell lines was tested. It is described here, since it deals with and included the same strains *14p* and *T-333* of Experiments I and II. In this instance, duplicate cultures both of the normal strain and the malignant cells were set up in *fluid culture media only;* no plasma was used. The fragments selected for cultivation were placed directly on the glass wall of the roller tube and wet thoroughly with the fluid medium 4-1-5-0 (40% B. S. S., 10% B. E. E., and 50% Cd. S.). In order to insure good adhesion to the glass, the tubes were allowed to stand upright for about one-half hour before the roller tube incubation. The surface tension of the thin fluid pulls the fragments tightly against the glass wall to which they soon become attached. Two days following the transfer of these cultures to this fluid medium, the fluid was renewed and one drop of a 10^{-4} dilution of virus suspension was added. Subsequent tests of this particular virus suspension showed that it had a low titer and that there were less than ten embryo LD_{50}'s per drop when this 10^{-4} dilution was used. However, the malignant cultures were infected by this inoculum of virus.

Following the addition of the virus the normal cultures were observed on each day to be in excellent condition and showed no apparent cell destruction. On the sixth day the normal cells were reinoculated with one drop of a 10^{-3} dilution of the same virus pool. The normal rat fibroblast cultures remained in an apparently healthy condition for over a month and a half thereafter. In the malignant cell cultures the first evidence of cell destruction appeared on the second day after exposure to virus and was severe by the fourth day at which time they were discarded. The colonies were destroyed and only a few active cells were present. As in the first two experiments, a large amount of virus was present in the supernatant fluid harvested from the malignant cell cultures. Furthermore, no virus was recovered from the normal cells.

Effects of Varying Amounts of Virus

Experiment X (Chart 2)—This experiment was designed to test the effect of varying amounts of virus on the normal *14p* and its malignant cell derivative *T-333*, both of which had been grown in the fluid culture medium (4-1-5-0) for one month beforehand. They were maintained in this medium throughout the experiment.

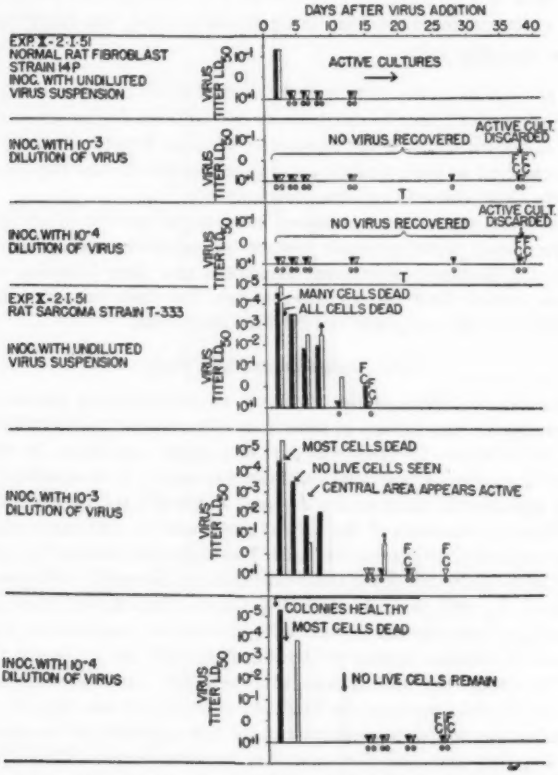


CHART 2. A comparison of the susceptibility of the two strains (resistant and susceptible) to varying inocula of virus. $\frac{F}{C}$ indicates assay of fluid and cells. T = transfer.

Throughout the experiment the normal cell colonies were larger than those of the tumor cell derivative *T-333*, but the total bulk of the cells was estimated to be about the same at the time of virus addition. Each inoculation was done in duplicate into tubes with four colonies per tube, one pair receiving undiluted virus suspension (10^6 LD_{50}) and the other two groups receiving a 10^{-3}, and 10^{-4} dilution of virus suspension respectively. The virus was added two hours following renewal of the medium. In all cases, 100 units of penicillin G (Merck) were also added at the time of virus addition.

During the entire period of observation, the normal strain *14p* appeared to be unaffected by any concentration of virus that was added while the malignant cells were observed to be destroyed. Figures 1 and 2 illustrate the destruction of this malignant cell strain by virus.

Virus was added in the same concentrations to the malignant cells, but two days after the normal strain had received virus. This was done so that the normal cell colonies would be comparable in size to the more rapidly growing malignant cell colonies. The highest concentration of virus destroyed many malignant cells within 24 hours; whereas, those cultures inoculated with less virus still showed healthy colonies at this time. Very severe destruction was apparent in all of the malignant cell series by the third day. All the destroyed cultures yielded titers of 10^{-4} to 10^{-6} LD_{50} of virus in the supernatant, whereas no virus was recovered from the normal cells. The condition of all of the colonies was followed carefully over a period of 27 days from the time virus was added. Only one group of cultures, those receiving the least amount of virus, showed a few questionably active cells in a innermost translucent zone of the tiny central areas of the colonies at the end of the first week. Thereafter, all of the cultures of *T-333* became progressively darker and had no apparent viable cells from the 11th day on. However, in some of the colonies, the malignant cells are probably not completely susceptible, since some living cells were observed for about one month.

Survival following heavy destruction—In a following experiment, a 10^{-3} suspension of virus was added to one of the control tumor cell cultures of *T-333* with the same cultural history as the group above. Despite initial severe destruction, some cells survived and eventually produced active continuous cultures despite the presence of virus, which persisted for 215 days. Subsequently, this cell line recovered and *two years later* was found to be still highly susceptible to the virus.

The effect of a continued high concentration of virus on the normal strain 14p cultures was then studied. Colonies of the susceptible tumor cells were introduced into six tubes already containing normal cell cultures. The tumor cell fragments were placed opposite the normal colonies. A 10^{-3} virus suspension was added three days later when the tumor colonies were growing actively. Two days later titrations of fluid from all these cultures showed values varying from $10^{-3.5}$ to $10^{-5.3}$ LD_{50} of virus. Virus persisted at 10 to 100 LD_{50}'s for more than a week in five of the tubes. Very severe damage of tumor cell colonies was apparent within 48 hours and increased during the first week of observation. The normal cell colonies in the presence of the high concentration of virus continued to grow and proliferate during the first week. Some malignant cells were scattered by the fluid and finally were re-implanted in the normal cell areas.

Strains with Intermediate Susceptibility

Intermediate between the high susceptibility of the malignant strain *T-333* and the very resistant normal strain *14p* is the reaction of a whole group of rat cells which were inoculated with virus. We have studied particularly the malignant cell strain *TSAT-624-72* which is from the same host as the above pair of strains. It is partially destroyed by virus but with continuous cultivation the surviving cells in the colony grow out abundantly. Many of these are in turn destroyed. Thus, there appears to be a kind of balance so that many new cells produce new virus; such cultures of *TSAT-624-72* have shown that

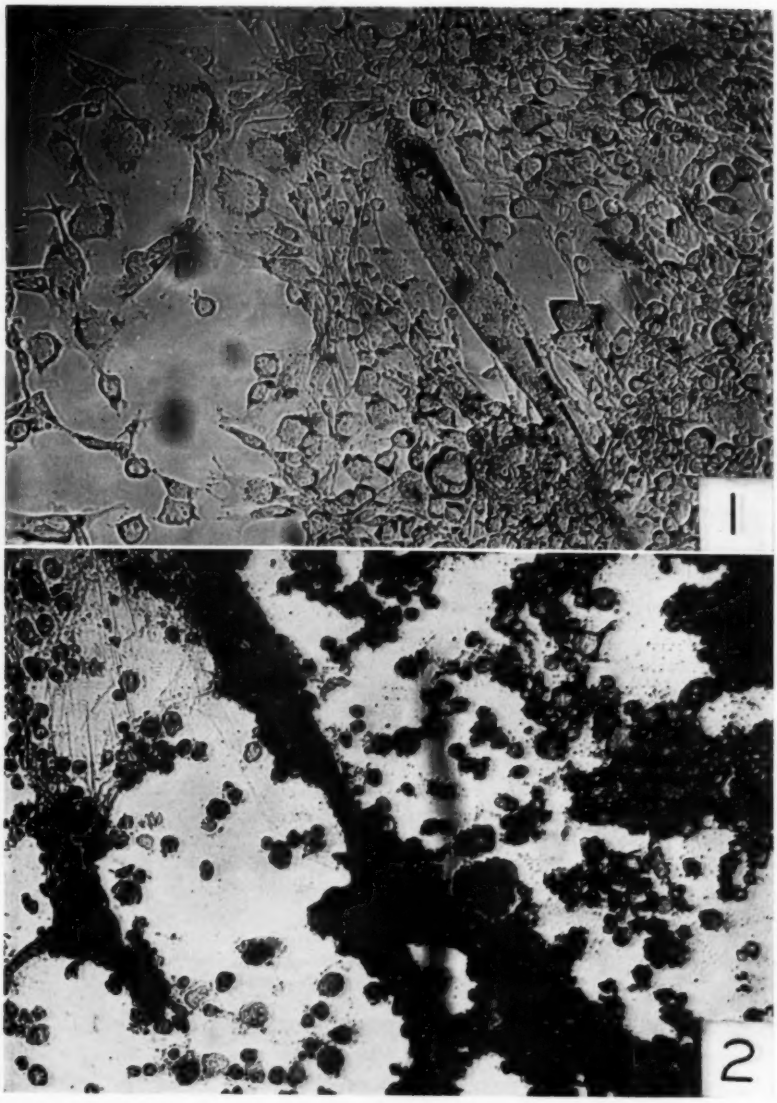


FIG. 1. Control roller tube culture No. 1791 of T-333 shows thin spread of active healthy tumor cells, with some giant cells. Many dividing cells. ×120.

FIG. 2. Virus infected culture No. 1792 of T-333. Eleven days following virus addition. Only dark cell debris and a few partially destroyed cells remain. ×120.

they can produce virus for many weeks and probably indefinitely under the right conditions.

Experiment VI-A (Chart 3)—Two roller tube cultures, #5, and #6 were selected from a group that had been maintained in 50 per cent human placental cord serum in balanced salt solution and grown directly on the glass wall for 125 days. These were changed to 70 per cent serum thirty-eight days before virus inoculation which in turn was added six days after the last renewal of fluid. They were maintained in 70 per cent serum for the rest of the experiment. Virus was recovered from both tubes during the first few weeks. Culture #5 was in poor condition on the 40th day after virus was added, so that only the granular calcified remains of the old colony areas with a few dark cell casts and cell debris could be found. On the third and tenth day after this observation and in spite of this lack of observable active cells the culture was continued and virus was found on the 43rd and 50th days. On the 29th day following the virus inoculation the handicapped culture #6 was

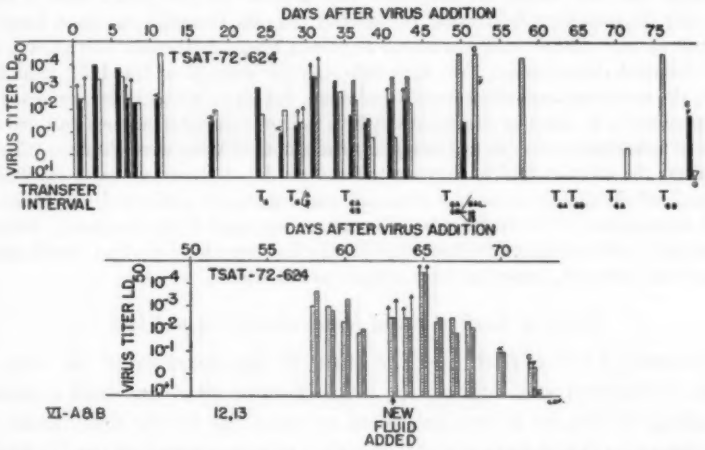


CHART 3. The continuous production of virus by a cell line of intermediate susceptibility. Compare with Plates III and VI. Exp. VI-A—Black bars = tube #5; White bars = #6 or #6A; Diagonal hatch = #6B. Exp. VI-B—White bar = tube #12; Barred bar = #13; Ascending arrow = greater than titer shown; < = less than titer shown.

transferred (T) and subcultured into two, 6A and 6B. Thereafter for a total of 78 days both cultures were carefully studied for cell destruction. During this period culture 6A was transferred (T) to new roller tubes five times and showed very active colonies with many dividing cells. Virus was present in the supernatant whenever tested, cell casts and degenerating cells which varied somewhat in number were found scattered among the healthy cells. This culture was interrupted because of a poor transfer 78 days following addition of virus, but virus had persisted until this time.

On the 50th day, culture No. 6B showed extensive outgrowth throughout the tube and some colonies were transferred (T) to make tubes No. 12 and 13 for another experiment. The colonies remaining in the tube (6B-Bal.) were kept growing and virus was found on the 71st and the 78th day. During this interval, the cultures remained in excellent condition and two further transfers were made. However, degenerating cells produced by growth of virus were continually present. Further growth was interrupted on the 78th day because of a transfer to a poor medium.

Effect of Cell Nutrition on Virus Yield

Experiment VI-B—In order to study the role of the supporting fluid medium, the following experiment was made. Cultures No. 12 and No. 13 (Chart 3) which had been infected with virus fifty days before were used for this experiment. These cultures were given two fluid renewals which stimulated a healthy outgrowth covering one-half the tube. Scattered cell debris and degenerating cells apparently produced by virus were constantly present. The culture fluid (4-1-5-0) was renewed and increased to 15 drops instead of the usual 10 drops so that the removal of a single drop of fluid from each culture for six days would not markedly exhaust the medium. On the sixth day, ten drops of new fluid medium were then added to the remaining culture fluid, and the cultures were then allowed to degenerate progressively without further fluid renewal. This took nine more days.

During the first half of this observation period there was apparently sufficient culture fluid available to permit the cultures to grow to a point where the colony areas spread throughout the tube. Many dividing cells were present. At this point, when it seemed likely that the remaining fluid would not nourish the cells favorably for much longer, the ten drops of new culture fluid was added to permit longer cultivation and allow a study of the eventual degeneration. Two days following the renewal of the fluid, most of the cells in the overpopulated tubes were rounded up, and approximately 50 per cent of the cells appeared to be dead or degenerating. It is unlikely that this was entirely the result of lack of nourishment and at this time, larger amounts of virus were obtained (Chart 3). Thereafter, the colonies rapidly degenerated and produced numerous hard, calcified, cell casts and cell debris. An occasional expanded and apparently active cell was found after careful examination of the entire tube. The culture appeared to be completely destroyed, with no active cells present, five days following the last renewal of medium. Small amounts of virus were, however, present as long as four days thereafter.

Effect of Lack of Fluid Renewals on Virus Yield

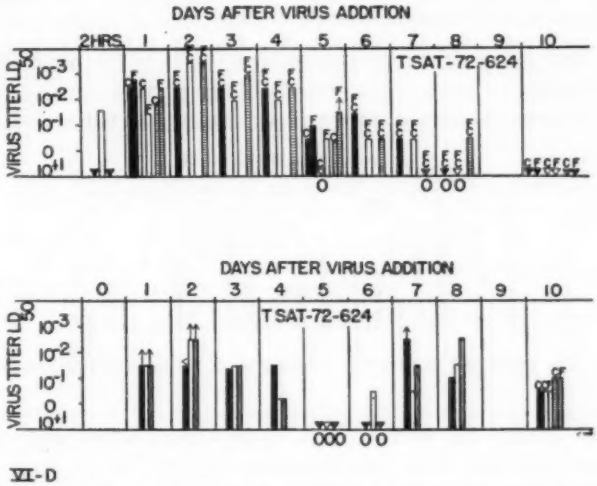
Experiment VI-D (Chart 4)—The effect of degeneration of the cells due to lack of new nutrient fluid on the production of virus was then studied by comparing the results in two groups of cultures. (a) In the first, *Series A*, a large series of cultures were inoculated with virus simultaneously and individual cultures were sacrificed at intervals. Virus assays were made of cells and fluid. (b) In the second type of experiment, *Series B*, three cultures were inoculated at the same time, and virus measurements were made on the fluid from each culture each day for the next ten days. In order to do this, fluid was then renewed each day.

Series A.—A group of 42 roller tube cultures was set up as follows: Three stock roller tube cultures, which had been cultivated by Mrs. Kubicek for over a year in three different types of media, one in 50 per cent human placental cord serum, another in 4-1-5-0, and the third in 1-1-4-4, were selected, since no single cultured group at the time was sufficient to start the experiment. These were expanded to thirteen, and grown in the 4-1-5-0 fluid medium. The colony fragments were mixed repeatedly in a syringe so that the final pool of fragments for the group of 42 cultures would be randomized. Five drops of this pool and seven drops of the 4-1-5-0 fluid medium were added to each of 42 tubes. Each tube was also treated with 100 units of penicillin and 100 units of streptomycin. The cultures were grown for seven days with three fluid renewals, but without antibiotic treatment. A 10^{-4} dilution of stock virus suspension was added, and the same antibiotics again added.

Tests for virus were done on three cultures, two hours after inoculation and incubation. The combined tissue and undiluted fluid from two cultures were negative. Fluid from the third tube titered to $10^{-1.5}$, whereas, the tissue showed a value of $10^{0.3}$ (i.e., it killed two of three embryos with the undiluted fluid).

In order to determine survival of virus in the incubated culture medium without tissue, a series of eight tubes containing the 4-1-5-0 culture medium alone were inoculated with one drop of the same virus suspension used above. Thereby, the virus suspension was diluted 1/10 in the medium. When the culture medium was tested for virus after two hours' incubation in the roller drum, some of the tubes yielded virus, but only with the undiluted culture fluid.

The cultures of *Series A* were observed daily. At the time the virus was added all showed active healthy colonies with many dividing cells. In the presence of the virus (Chart 4)



VI-D

CHART 4. The effect of nutrient fluid *renewal* on virus yield. Upper row represents the amount of virus obtained from cells when fluid was not renewed. Lower row represents virus yield from identical cultures, but with fluid renewal each day. $\frac{F}{C}$ = assay of fluid and cells.

all grew progressively for at least six days with an extension of the growth as a thin monocellular spread throughout the tube. During this time, both the control and virus infected cultures showed some scattered degenerating cells in most microscopic fields. On the seventh day, the group to be sacrificed had ten or more degenerating or dead cells in most of the low-power microscopic fields, but there were many dense areas of new growth. Many cells were, however, rounded and only a few dividing cells could be found. On the eighth day, the group to be sacrificed showed extensive degeneration of the cells with about 50 per cent of the population dead. Some apparently healthy expanded cells were still present. At ten days, at the time of the last viral assay, all tubes showed chiefly degenerating cells and cell debris with occasional tiny clumps of apparently active cells. There were also some sparsely distributed single expanded cells. No virus was found at this time. In the group sacrificed on day eight and day ten, those tubes (Chart 4) which showed no virus did however show some active expanded cells.

Series B (Chart 4)—In this experiment, three tubes were treated daily with new fluid media. On the day following the addition of virus, the three experimental tubes and the control showed active healthy colonies with many dividing cells. From the second through the eighth day following the addition of virus, all of the cultures grew rapidly. By the seventh day, there were dense spreads of cells throughout many areas of the tube. All of the cells were fairly clear and a great many dividing cells were present. Throughout this entire period, both the experimental and the control cultures showed a few scattered degenerating cells per microscopic field. It is interesting to note that two of the cultures on the fourth day yielded very little virus and all three on the fifth day yielded none. Again on the sixth day, only one culture yielded a little virus. However on the seventh and eighth days, when the cultures were still in excellent condition with cells densely packed together, a significant increase in virus was obtained. On the tenth day, all of the cells seemed to be dead in one culture, while the other two were still apparently active and healthy with many dividing cells. All three cultures had little virus.

The frequency of fluid renewal undoubtedly affects the physiological state of cultured cells. To study the effect of this on viral susceptibility we withdrew fluid treatments from one pair of previously infected cultures (Exp. VI

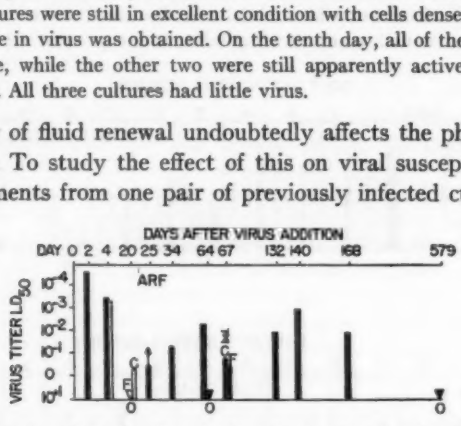


CHART 5. The continued production of virus by a normal rat cell line in continuous culture.

B, Chart 3). During the first six days, despite some withdrawal of nutrient, the cells grew well and virus yield decreased slightly. The addition of a larger amount of fluid than previously added produced increased cell growth and was accompanied by an increase in virus. Subsequently the undernourished cells degenerated. Virus yield decreased.

In a second experiment (Exp. VI-D; Chart 4) on the same cell strain, the effect of fluid renewal was directly compared in two series. The cells were infected at the start of the experiment. In series A, a large number of cultures were infected. No fluid was renewed. Cultures were observed and sacrificed at daily intervals. In Series B, fluid was renewed daily, and virus measured in the fluid. The experiments support the concept that actively metabolizing cells are necessary for virus production (1) and that degenerating cells yield little virus. However, frequent renewal of culture fluid which induced very rapid cell proliferation did not produce a concomitant increase in virus yield.

A Normal Rat Cell Strain With Intermediate Susceptibility

Experiment XI Normal Rat Strain—ARF (Chart 5)—The susceptibility of another cultured strain of normal rat cells related to 14pf (the normal resistant

fibroblast strain) was determined. This normal strain of chondric origin was derived from the articular ridges of the femur of the same rat as that which furnished our 14pf strain.

A duplicate set of cultures was set up for virus treatment. A dilution of 10^{-4} of stock virus was added to very active cultures two days after transfer. These were destroyed within two days and showed marked degeneration with the production of large masses of cell debris and large amounts of virus. A series of transfers were then made and the yield of virus determined at intervals. The amount of degeneration fluctuated slightly in the early part of the experiment, but later decreased to a minimal amount of cell degeneration. Accompanying this, a small amount of virus was continually produced, and the last positive test for virus occurred on the 168th day.

The cultures were maintained for an additional period and new virus added a year and a half later. On this occasion, again, the very active cultures were almost completely destroyed two days following the addition of a 10^{-3} dilution of virus, and a high yield ($10^{-6.8}$) of virus was obtained. In spite of severe degeneration and the presence of virus, the few remaining active cells recovered to form new colonies. As the degeneration progressed, there was formed about the colonies a dark, necrotic ring inside of which one could see the

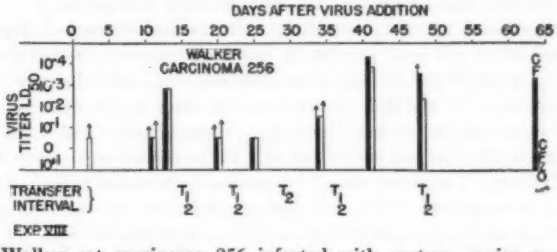


CHART 6. Walker rat carcinoma 256 infected with eastern equine encephalomyelitis virus. T - Transfer of tube 1 or 2 or both.

translucent central area of still active cells. The experiment was discontinued once the recovered cultures had again regained their original growth rate.

This normal strain makes an excellent one for the study of recovery following viral injury and cell destruction, since there is a rapid replacement of the degenerating masses of cells with active cells. The cell destruction leads to the formation of a dense, necrotic, calcified ring around active, translucent, central areas of the colonies, quite similar to the results observed with strain *T-333*.

Walker Rat Carcinoma—256

Experiment VIII (Chart 6)—The susceptibility to this virus of a well established transplantable rat carinoma, the Walker rat carcinoma 256, was then studied. This 8-year-old cultured line of tumor cells had been grown in 1-1-4-4 substrate plus 1-1-4-0 supernate before the experiment was set up. A few cultures were then transferred to the fluid medium 4-1-5-0. Two days following the transfer to this fluid medium, duplicate cultures were treated with a drop of virus suspension (10^{-4}). The cultures continued to grow actively

for two days. However, on the fourth day, cell destruction was very severe with about 95 per cent of the cells destroyed and yielding masses of dark cell debris with a few scattered active cells between. Subsequently, despite the presence of virus, the surviving active cells repopulated the roller tubes. Recovery of cells continued with production of new virus, and on the fifteenth day following virus addition both cultures were transferred. At this time, the entire roller tube was covered with active healthy colonies, and much of the original cell debris had disappeared. On the day following this transfer, cultures were observed to be in excellent condition with little evidence of cell debris or new cell destruction. However, five days following transfer, scattered single degenerating cells were observed.

A third transfer was necessary on the 22nd day after the original addition of virus and seven days following the last transfer. Again, the cultures showed very good growth and continued to produce virus. Cell destruction recurred and became most severe seven days later, at which time about 50 per cent of the cells were destroyed. Again, the cultures recovered, and it was necessary to make an additional transfer at day 36. Following this third transfer, fairly large active healthy colonies were obtained, but on the fifth day following transfer, they appeared to be more susceptible to virus than any earlier period. By the seventh day following transfer, the intact masses were surrounded by dark cell casts and cell debris. Again, the cultures recovered well and formed large colonies making possible an additional transfer on the 48th day following viral addition.

Following this fourth transfer, one of the cultures showed marked degeneration with only a few small active cell areas present. In this case, recovery was not possible; the culture died slowly. On the 55th day, only a few scattered expanded cells were present. A final assay of degenerating cells and fluid of this tube was made on the 64th day and virus was recovered. The other culture, however, continued to remain active with scattered degenerating cells throughout the tube and active cells only in the central area, which was surrounded by necrotic cell debris. A recovery period followed with increased spread of active colonies in the presence of much cell debris and degenerating cells. On the 64th day, virus was obtained from this relatively healthy culture. It was sacrificed for assay of cells and fluid for virus. It probably could have been cultured continuously in the presence of virus.

The response of this strain to virus is characterized by the continued presence of virus and a recurrent extensive destruction of the cultures followed by repopulation of the culture tube by the surviving cells.

Tests For Viral Variation

We next desired to test the possibility that the virus might have changed in its ability to destroy cells while in culture. Virus which had been grown in cultures of this carcinoma, *WRC-256* for 53 days was compared with the standard virus.

The tissue culture adapted virus was passed once through chick embryos in order to inoculate new cultures with comparable amounts of virus. Two pairs of cultures of Walker Carcinoma 256 were tested. Similar destruction was observed throughout the period of observation in all cultures and about the same amount of virus was obtained from each (Table I). However, these titrations were done by making 100-fold dilutions, which were inoculated on embryos, instead of the usual ten-fold dilutions. Thus, there is a higher standard error in the titrations.

A further test of the same virus obtained from tissue culture, (*WRC-256* adapted) was made on an entirely different group of rat cell cultures. These were *ANSAT 21* and *TSAT 2936*, the latter an animal passage tumor cell strain derived from *ANSAT 21*. Duplicate cultures of each strain were inoculated. The tissue culture adapted virus produced the same amount of cell destruction in the cultures as did the standard virus, and a similar large yield of virus was subsequently obtained from the two cultures. However, the tumor cell line *TSAT 2936* was less susceptible to both virus inocula (Table I) than the altered normal line *ANSAT 21*.

In two separate experiments tests for variation in the destructiveness of virus grown for fifty-three days in a tumor cell strain were made. The virus from tissue culture destroyed the same number of cells as did the standard chick embryo virus.

TABLE I

Amount of Virus Recovered from Cultures of WRC-256 and ANSAT-21 Following Inoculation with Tissue Culture Virus and Standard Chick Embryo Passage Virus

TISSUE CULTURE	VIRUS	DAYS AFTER INOCULATION	
		3	6
		Virus Titer LD_{50}	
WRC-256	Tissue culture	4.5+	5.0
		4.5+	5.8
	Standard	4.5+	5.0
		4.5+	5.8
ANSAT-21	Tissue culture	5.5+	5.0
		5.5+	5.0
	Standard	5.5+	5.0
		5.5+	6.5+

Human Cell Strains

Tests of the susceptibility of two human tumor cell strains were then carried out to compare their susceptibility to that of the rat cells. The first cell line *D-1 Re* originally derived from a human chondro-myxosarcoma in 1931 and subsequently passed through a rat, was found to be highly susceptible to the virus. The second line tested was strain *A Fi*. It has been in continuous cultivation since 1935 when it was explanted from a human fibrosarcoma. It was also found to be susceptible.

Experiment VII-D-1 Re (Chart 7)—The cultures used were obtained from tissue which had been maintained previously in medium 1-1-4-4 substrate plus 4-1-5-0 fluid. It was transferred in March 1950 to a medium of 40 per cent balanced salt solution, 10 per cent bovine embryo extract and 50 per cent human placental cord serum. A 10^{-4} dilution of virus was added two days later when the colonies were fairly large. Tests for virus two

hours after addition were negative, but a high titer was found at two days. At this time, the infected cultures showed rounded granular cells with none dividing. This was taken as evidence of viral destruction. Five days later very few intact cells remained. Only cell debris and dead cells were present on the fourteenth day. Uninoculated controls remained healthy. The largest amount of virus was obtained on the second day, when both cultures with tiny colonies were transferred. From this time on, the cultures gradually degenerated and were discarded on the 33rd day, at which time they showed only tiny, dark, necrotic cell masses and some tiny translucent cell areas in the center of the remaining colony remnants. At this time, no virus was recovered.

Experiment VIII (Chart 7) also included strain *D-1 Re*. These cultures were derived from the control cultures of the above experiment. Colonies were transferred on April 25, 1950, and two days later, two cultures were treated with new fluid, and a drop of virus (10^{-4}) was added to each. The cultures, at first, were in excellent condition, but two days

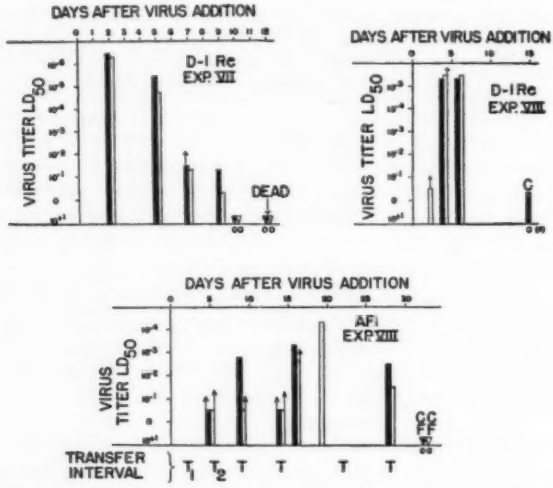


CHART 7. Infection of two human tumor cell lines with eastern equine encephalomyelitis virus.

later some showed rounded up cells and none was found dividing. On the fourth day, destroyed colonies and cell debris were present. Subsequently, most cells were completely destroyed with occasional cystic cell remnants. One culture was held for fifteen days and very little virus was found in the destroyed cell debris (Chart 7).

Strain A Fi (Chart 7)—The susceptibility of this tumor cell line was tested simultaneously with strain *Di Re* in Experiment VIII. This cell line had been grown in 1-1-4-4 substrate plus 1-1-4-0 fluid and was transferred to 4-1-5-0 fluid medium on April 25, 1950. Two cultures were infected on May 10, 1950, with a 10^{-3} dilution of virus, five days following transfer. One day and two days following virus addition, the cells were observed to be in good condition. By the fifth day, there were many degenerating cells with a few tiny colony areas showing an outgrowth of healthy cells. At this time, the first test for virus showed that all embryos inoculated with undiluted supernatant fluid were killed by virus. The mixture of degenerating and healthy cells persisted until the ninth day.

These experiments indicate that two human tumor cell strains which had been maintained for more than fifteen years in continuous culture were destroyed either partially (A Fi) or completely (D-1 Re) by this virus. Their susceptibility is comparable to that of the rat tumor cell strains.

RESULTS

The destructive effect of the virus of eastern equine encephalomyelitis upon one cultured line of rat cells *T-333* and the relative lack of effect of the same virus upon another cultured line of rat cells *14p* is shown best by an examination of the cells in the roller tube cultures. Several days after infection the loosely bound masses of dark cell debris with remnants of shrunken inactive cells which appear in cultures of the cell of malignant derivation *T-333* (Figs. 1–2) are clearly different from cultures of the highly resistant normal cells. The latter show no change in the normal clear smooth sheet of thin extended cells, even when inoculated with virus titering as high as 10^{-5} or 10^{-6} on the chick embryo.

Associated with the marked differences in the effect of the virus of the cell lines are the comparative amounts of virus obtained from the supernatant fluid or from homogenates of the cells and the fluid. Cultures of the susceptible tumor cell yield large amounts, while the insusceptible normal cell cultures show little evidence of virus multiplication (Charts 1–2).

Six separate experiments comparing the susceptibility of cultures of the line of normal rat fibroblasts *14p* and derived tumor line *T-333* cultures have been carried out; five of them in roller tube cultures after outgrowth had occurred and one in drilled-slide cultures where there was little circulation of nutrient. All comparisons have shown the cultured line of malignant cell derivation to be much more susceptible to the virus. The resistant normal cell remained resistant, even when placed in 1 per cent trypsin (13) for one hour and then exposed to the virus. This was true in two experiments in which a number of other cell lines also grown in the same medium were shown to be susceptible.

An extreme test of the relative susceptibility of strain *14pf* and *T-333* cells was carried out by placing colonies of the susceptible strain of malignant cell derivation in tubes containing colonies of the insusceptible normal cell. The susceptible colonies were placed on the opposite side of the tube from the colonies of the resistant cell and could be distinguished morphologically from them. A drop of chick embryo suspension diluted to a point where it contained 50 to 100 chick embryo LD_{50}'s of virus was then put in each of the six tubes containing this combination. In all of these, malignant cell colonies were rapidly destroyed while no effect was apparent on the normal cell during the first few days.

In this study we have tested sixteen cultured cell strains and find that in general susceptibility is not dependent upon the malignant nature of the cell. Normal cell strains may also vary in their relative susceptibilities. A normal rat fibroblast strain *M*, derived many months previously from muscle, was destroyed as rapidly by virus as was a human tumor cell line (Di Re) (Chart 7). Both yielded large amounts of virus.

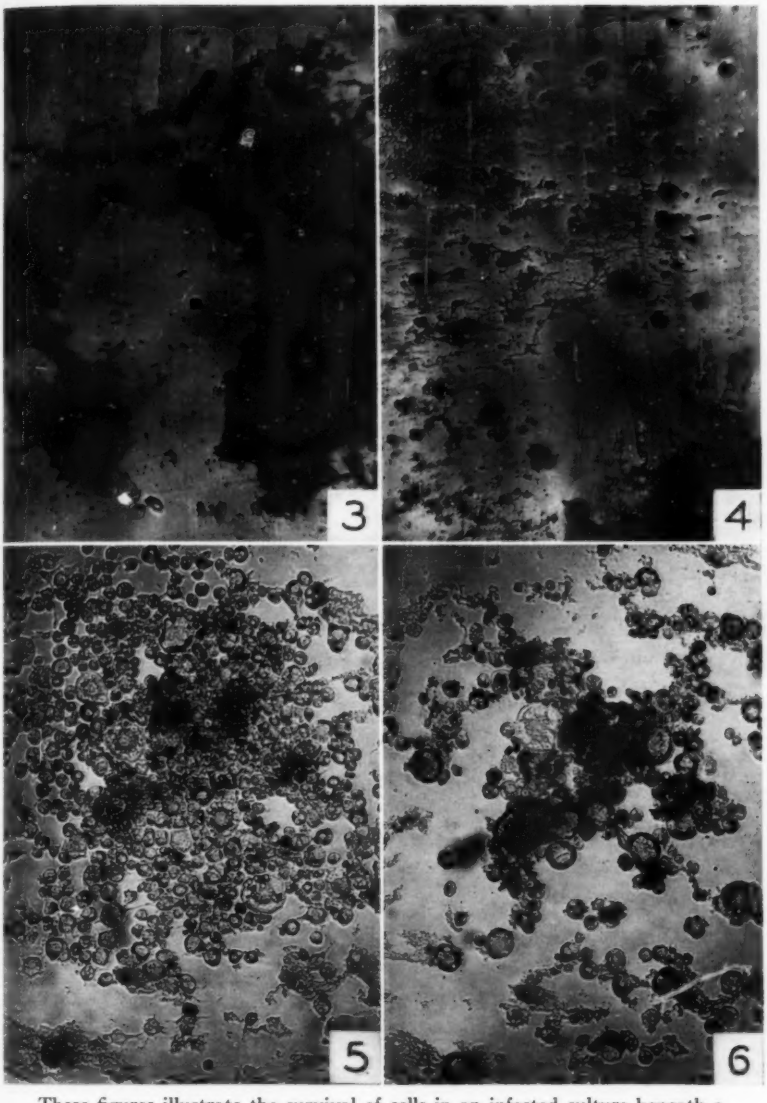
It is almost impossible to tell at any given time if all of the cells in a virus infected culture are actually dead (Figs. 3–6). With continued cultivation, living cells may sometimes migrate from underneath a calcified opaque mass of cell debris. The forced removal of such a tiny hard mass from the glass wall may uncover susceptible cells (Figs. 4 and 5).

A study of some of the cell strains showed further that an intermediate type of susceptibility may occur in cultures and be characteristic for these strains. In these cases, virus growth and cell growth obtain simultaneously. Some cells may be destroyed (Figs. 7 and 8); virus in small amounts is continuously present in the supernatant fluid bathing the cells (Chart 3). Cell multiplication continues at a good rate, so that the colonies must be transferred, and the nourishing supernatant fluid replaced. Since the cells continue to survive and multiply in the continued presence of virus, it is obvious that cell multiplication stays ahead of cell destruction due to virus multiplication.

Any explanation of this must take into consideration some estimate of the quantities of cells and virus particles present. If the infectious dose for the chick embryo is roughly the same as that for a roller tube (and several dilution experiments so indicate), then each milliliter of fluid within these roller tube cultures continuously contains 10,000 infectious particles (Charts 3 and 6). However, this is far fewer than the number of cells within an area of active cells which is estimated to be of the order of 10^6 cells per sq. cm. Thus it is possible that all cells are not exposed to the virus equally. An explanation of this peculiar balance between cells and virus production might be that the increase in the number of cells is more rapid than that of the virus particles parasitic on individual cells. Such an explanation has been proposed by Sonneborn for the loss of the parasitic killer factor in clones of paramecium (14). Without actual data on the rate of multiplication of the virus that we have studied under these specific conditions and the possible unequal distribution of the virus in the culture, this remains theoretical.

Electron Microscope Observations

Previous studies (6) of chick embryo fibroblasts destroyed by the virus of eastern equine encephalomyelitis were not extensive enough to yield any detailed morphological description of the process of cell destruction. We were able to find only remnants of destroyed cells surrounded by virus. The present



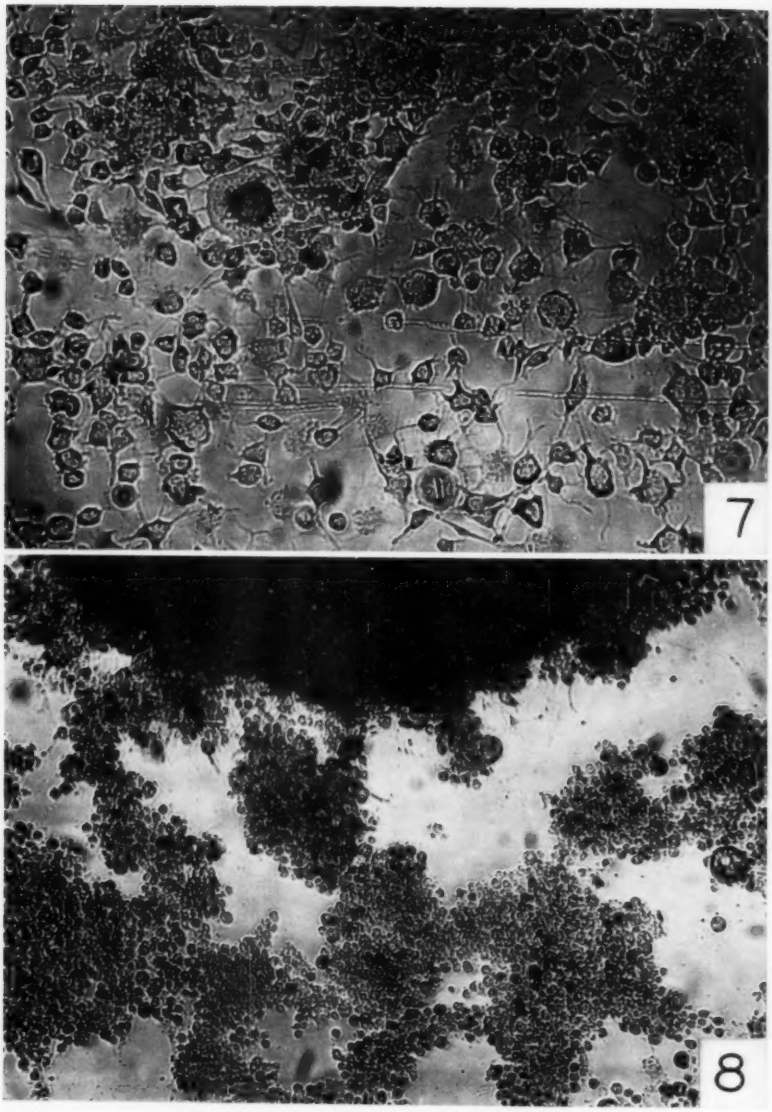
These figures illustrate the survival of cells in an infected culture beneath a destroyed mass of cells

FIG. 3. Hard calcified remnant of necrotic culture ring 78 days following virus addition. Minimal virus ($>10^{-0.5}$) present one month previously. No virus found one week previous. No active cells anywhere in tube, but suspected of being present beneath calcified ring. ×30.

FIG. 4. Hard ring forced to one side uncovered a small group of active cells (upper left). ×30.

FIG. 5. Higher magnification of Fig. 4. Most cells intact, others damaged. ×85.

FIG. 6. Four days after cells had been uncovered most of the cells were destroyed. One dividing cell present. All cells destroyed a week later. Viral assay negative on the 14 day after cells were uncovered. ×85.



Peripheral margins of TSAT-72-624 colonies

FIG. 7. Uninfected control. Photograph of roller tube with cells slightly shrunken. ×120.

FIG. 8. Colony destroyed. Only cell debris and rounded nuclear masses remain. Active cells immigrated from this and other colonies later. Six days following addition of virus. ×120.

studies have been of particular interest because we are able to see a variety of effects of this virus on cells. There may be (a) partial disintegration of one or two of the filamentous processes which grow from the cell (Fig. 9 and 10); (b) an array of virus particles imbedded along the edge of a cell (Fig. 11); (c) a partial destruction of a portion of the cell proper with the rest of the cell structurally intact (Fig. 12); and finally (d) a complete disintegration of the cell with only the barest outline of structure left. With these latter cell remnants, there may or may not be particles indentifiable as virus scattered among the debris (Fig. 13), often apparently buried in the small vesicular remnants of the cytoplasm. No particular direct effect on the nucleus has been seen.

A study of the process of cell destruction by this virus on living cells with phase microscopy (Fig. 14–16) confirms the piece-meal nature of the early effect of the virus on the cells. Nuclei are left apparently intact in the middle of the cellular debris which remains. However, the impossibility of observing this virus directly by phase microscopy prevents a complete interpretation of the virus action.

Furthermore, it would seem possible that each cell type has its own particular sequence of destructive effects. We cannot at present adequately correlate these changes within a given cell strain.

DISCUSSION

The problem of the increase in virus units, which follows upon the infection of the cell, has become such an important one involving so many fundamental concepts and different fields of endeavour, that it now is difficult to describe, without bias, what one sees in the electron microscope. It seems to us that certain observations concerning the virus units are definite. All units are not of equal size (Fig. 17) or density. Within a group of particles there may be at least a two-fold variation in diameter. Individual particles often do not lie free of the destroyed tissue but seem to be sitting on bits of cytoplasm which may be distended like balloons (Fig. 13). This appearance is similar to that produced by the effect of decreased osmotic pressure applied to cells by washing with water before fixation. It may then be that the cytoplasmic particles have been so changed by the virus that they now are rapidly distended by isotonic fluids.

Interest in the selective destruction of certain tumor cells by viruses was stimulated by a series of recent reports (15–18) that certain neurotropic viruses exert a degree of selective destruction of tumor cells in the host. In some experimental models, this leads to elimination of the tumor and recovery of the host (17). For this reason, we first compared the susceptibility of a normal rat fibroblast cell and one of its tumor cell derivatives (10). The latter was found to be highly susceptible and the normal cell relatively very resistant. That this

variation in susceptibility is not dependent upon malignancy was promptly established by the demonstrated high degree of susceptibility of a strain of slow growing normal rat fibroblasts derived originally from muscle. It is also well known that chick embryonic cells are highly susceptible to destruction by

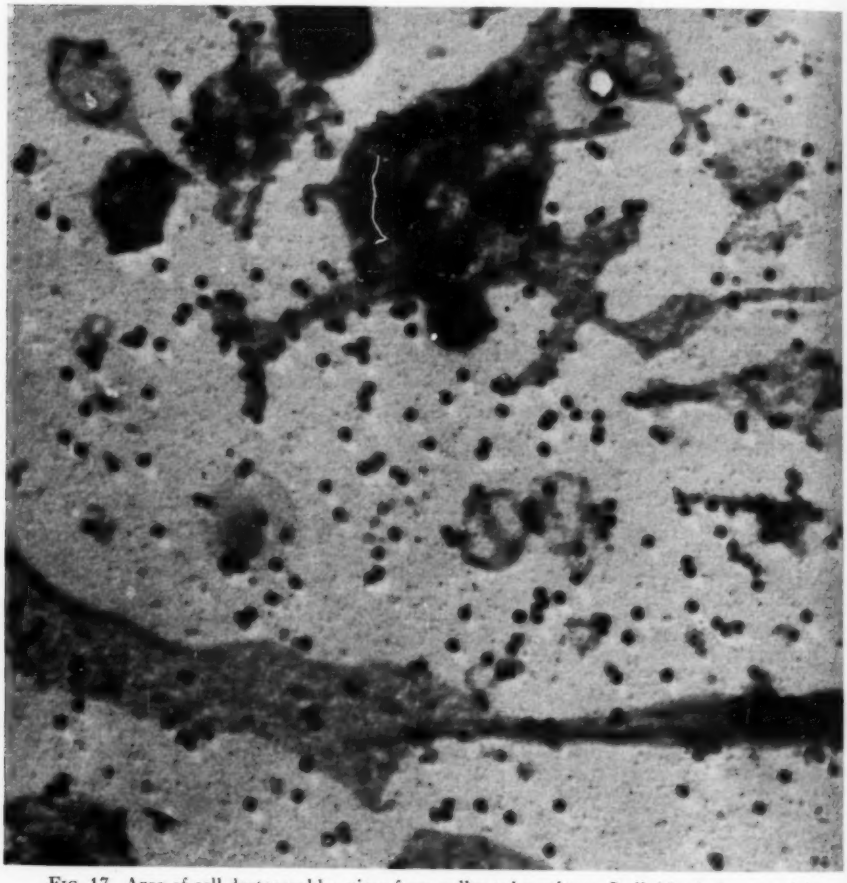


FIG. 17. Area of cell destroyed by virus from roller tube culture. Individual virus particles appearing in doublets and clumps show variation in size. Shadowed with chromium. ×35,000.

the virus (6, 7, 19); whereas, the adult chicken is only mildly affected (20). These differing effects of this virus on normal and tumor cells would in itself make it very difficult to apply the destructive effect of this virus in tumor therapy, even were a safe strain of virus found. It is of further interest that this

particular virus was one of the few found by Koporwski and Norton not to have a selective destructive effect on tumors in the mouse (18).

One human malignant cell strain is rapidly destroyed; whereas, another is incompletely destroyed.

SUMMARY

Roller tube cultures of thirteen established strains of rat cells derived from different types of mesenchymatous cells have varying susceptibilities to the virus of eastern equine encephalomyelitis. This variation is not in general dependent on the malignancy of the cell line, nor on a known degree of cell exposure, since all the cell strains were tested when grown on glass without clot. A normal cell strain and its specific malignant cell derivative of *in vitro* origin demonstrate extremes of susceptibility. Certain other strains show slight to complete destruction. Human tumor strains complete to severe destruction.

Several cell strains of intermediate susceptibility continue to support virus growth for periods exceeding four months and through a series of transfers from tube to tube without renewed virus inoculation or renewal of cells. The destruction of the cells in these persistently infected cultures may be focal in nature or so minimal that it does not greatly exceed that present in the uninfected cultures. The long continued presence of this virus in cultures of several lines of cells has shown no evidence of viral variation toward increased cell destruction. Loss of virus from continuous cultures previously supporting virus might be explained by a more rapid growth of cells than virus, yet inhibiting factors in the media used could also contribute to this phenomenon.

Electron and phase microscope studies show that degrees of cell destruction by virus exist. Portions of the cell are destroyed before complete disintegration occurs.

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PLATE I

FIG. 9. Destruction of individual cell process by virus. Virus particles may be seen more commonly in destroyed area. Roller tube culture. Fixed with osmic acid vapor. Unshadowed. ×20,000.

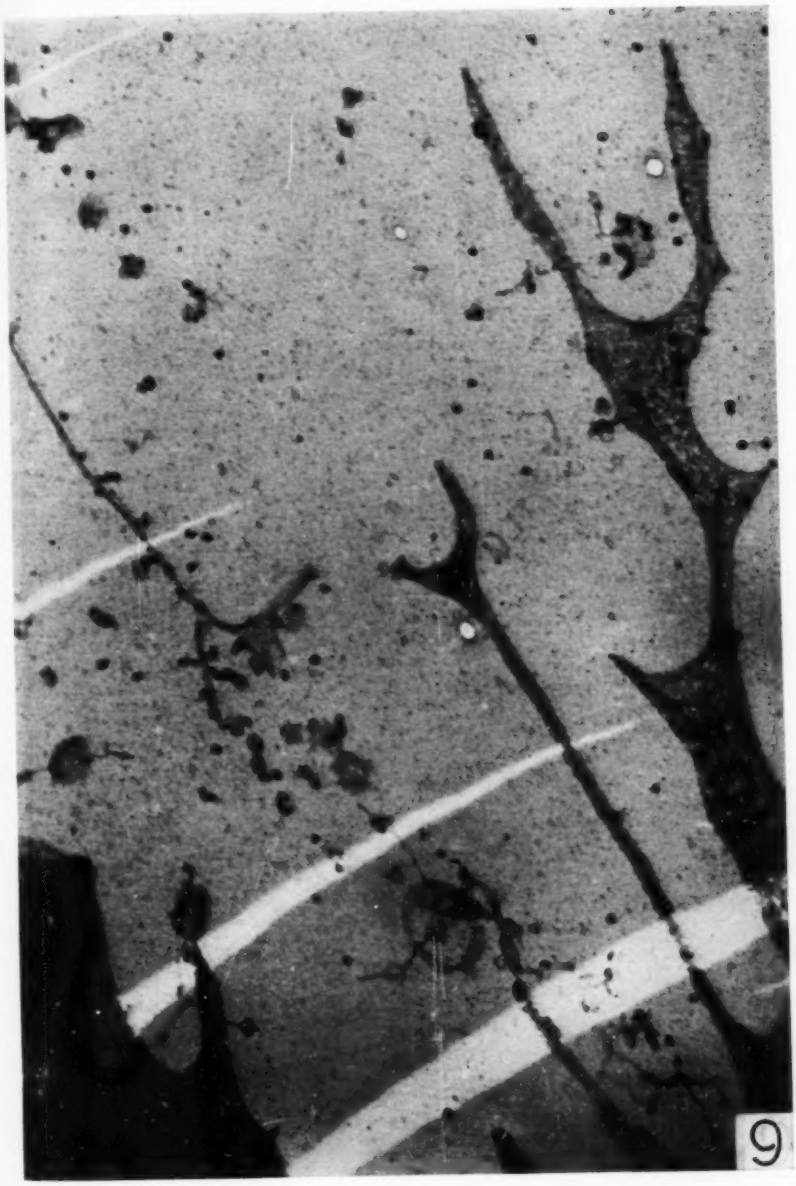
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PLATE II

FIG. 10. Another area of destruction present in same culture. Fixation and treatment same. ×20,000.

10

PLATE III

FIG. 11. Virus particles along edge of intact cell. It is unlikely that they are merely adsorbed here, for whole cell was intact and virus was not plentiful elsewhere. No shadowing. ×20,000.

PLATE IV

FIG. 12. Piece meal destruction of partially susceptible cell from mixed culture of 14p and T-333. One-half of cell extension is intact with typical fibrous structure previously described for this cell (21). Roller tube culture. Osmic acid fixation. Shadowed with chromium. ×15,000.

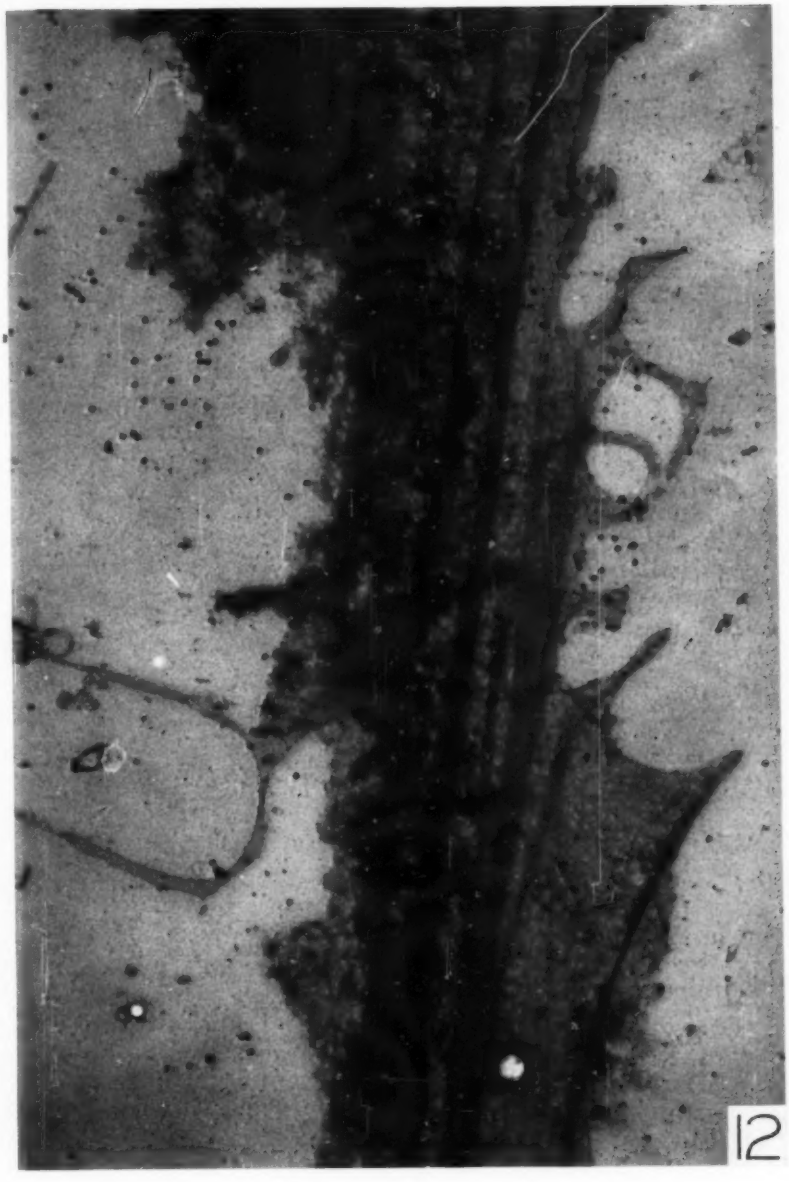
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PLATE V

FIG. 13. Area of destruction and cell debris with virus. Susceptible tumor cell. Ballooning disintegration of cell material, possibly including mitochondria. Drilled slide preparation. No shadowing. ×27,000.

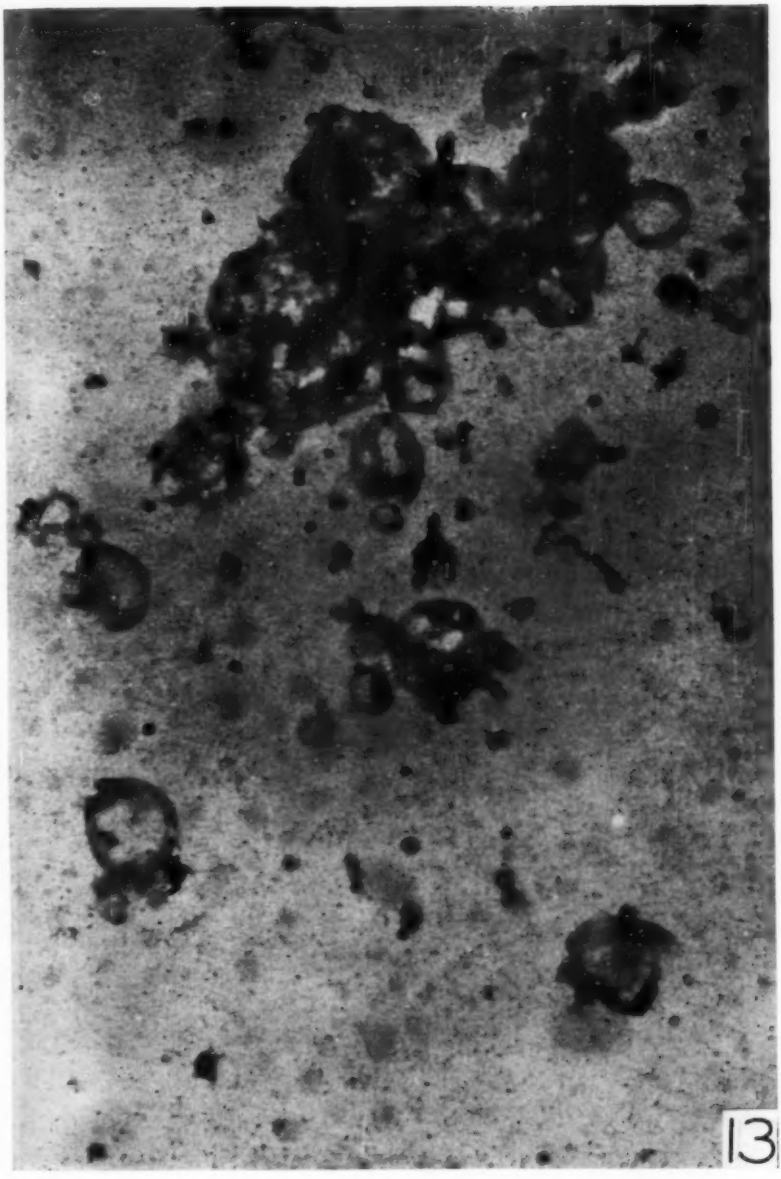
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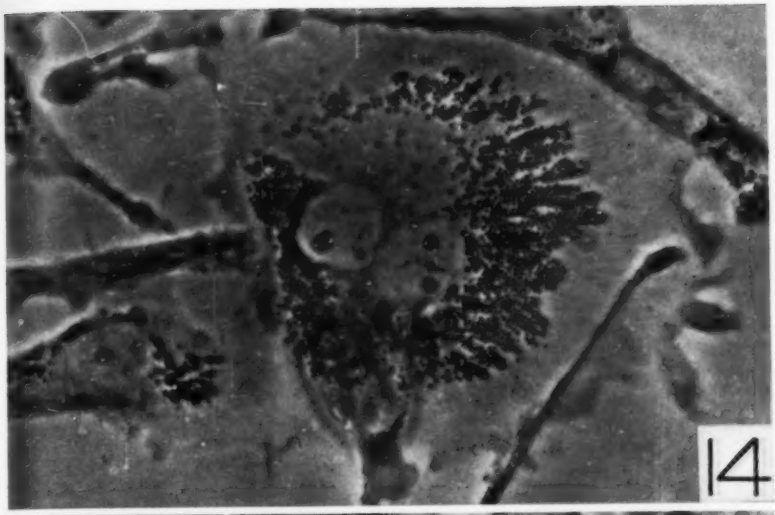
PLATE VI

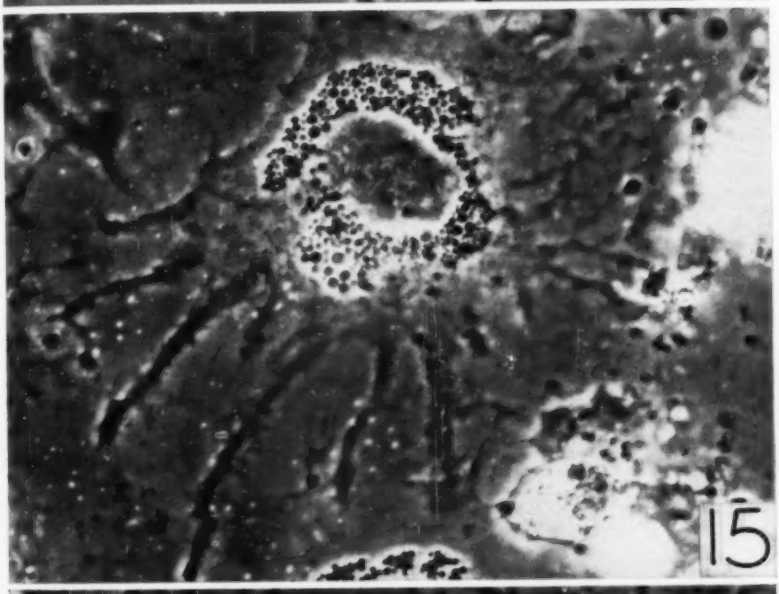
FIGS. 14, 15, 16. Phase microscope photographs showing cell destruction by virus. Cells from TSAT-72-624 (see Chart 4).

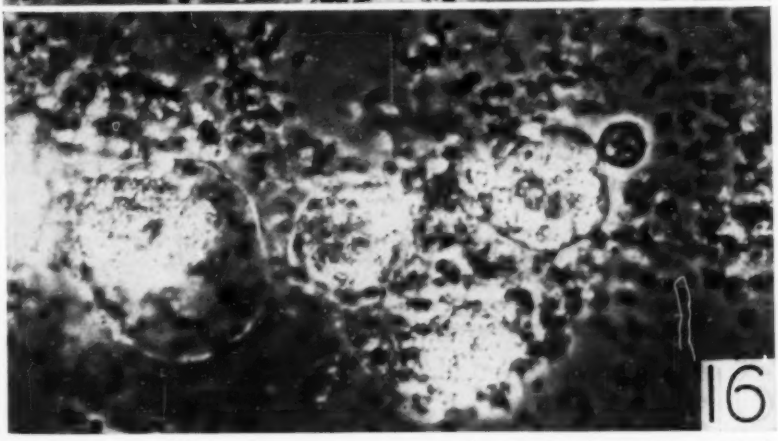
FIG. 14. Intact cells from control culture—no virus. ×730.

FIG. 15. Early evidence of cell destruction by virus. ×730.

FIG. 16. Cell debris by virus with relatively intact nuclei. Virus had been added to the culture 48 hours previously. ×730.







(Phase microscope photographs taken by Mr. Peter Sapranauskas).

THE EFFECT OF SPECIFIC ANTIBODY ON THE OXYGEN CONSUMPTION AND GROWTH OF *S. MARCESCENS*, *S. TYPHOSA* AND *SH. PARADYSENTERIAE**

RICHARD H. FOLLIS, JR., JACK M. BURNETT AND ZELDA E. LASCHEVER

The Department of Pathology, The Johns Hopkins University School of Medicine

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From time to time during the evolution of our knowledge of bacteriology and immunology, attention has been directed at the possible effect which specific antibody might have on the metabolic activities of various bacteria. As early as 1890 Charrin (1) had noted that the serum of vaccinated animals inhibited the production of blue pigment by *B. pyocyaneus*. A few years later Ghéorghiewsky (2) studied this question further, showing that there was no lysis or destruction of the organisms to account for this phenomenon. At the same time von Dungern (3) showed that sera of individuals infected with *B. anthracis*, cholera vibrio and staphylococci contained antibody which inhibited proteolytic enzymes produced by these organisms. In 1908 Ascoli (4) coined the term: "Antiblastische Immunitat," in the case of the effect of antiserum on *B. anthracis*, noting that immunity is dependent on an inhibition of the formation of a capsule which is a prerequisite for its successful development in the animal body. From then on a continuing series of contributions appeared dealing with the effects of specific antibody on a variety of organisms with relation to such activities as oxygen consumption, reduction of methylene blue, liquefaction of gelatin, inhibition of methemoglobin formation and growth (5–19).

Some preliminary experiments led us to reopen this question in the hopes that the utilization of certain refinements in technique such as purified (ethanol precipitated) antibody, simple substrates, and suspensions of washed organisms might be advantageous. Employing a series of nutrients such as glucose, succinate, malate, mannite, sorbitol and inulin, no differences of O_2 uptake were found when suspensions from 9 hour cultures of *S. marcescens* were observed in the presence of purified specific antibody in comparison with those metabolizing in the presence of purified antibody to some unrelated organism such as *S. typhosa*. However, when the O_2 consumption of organisms freshly inoculated into a conventional medium was studied, a definite effect of specific antibody was detected. There was a dramatic increase in O_2 consumption over that of the controls (20). In the present communication we should like to present

* Aided in part by a grant from the Division of Research Grants and Fellowships of the National Institute of Health, United States Public Health Service.

observations on other organisms and to detail further studies on *S. marcescens*. A summary has appeared elsewhere (21).

MATERIALS AND METHODS

The organisms used have been 4 strains of *S. marcescens* (JH, SC, GC, and UM), a strain of *S. typhosa* (H 901) and a strain of *Sh. paradysenteriae* (F 16).

Rabbits were injected intravenously 6 times at 2–3 day intervals with emulsions of live *S. marcescens* (JH) prepared from cultures grown for 24 hours on pancreatic digest agar. Similarly, other rabbits were injected with suspensions of typhoid or paradysentery organisms. Twelve days after the last injection the animals were bled from the heart. All sera were inactivated at 60°C. for 3 minutes and then titrated against respective live organisms. After finding the sera to be active (titer: 1–20,480 to 1–40,960) similar ones were pooled. A portion from each of the 3 batches was frozen. The remainder was treated by ethanol in the cold to obtain the *gamma* globulin fraction, utilizing the technique described by Cohn *et al.* (22). Normal sera were treated in a similar manner.

Measurements of O_2 consumption were carried out in duplicate in the Barcroft-Warburg apparatus. An appropriate amount of an overnight culture in trypticase soy broth was diluted with enough fresh trypticase soy broth to provide adequate media to fill each flask with 3 cc. The center wells contained .2 cc. 10% KOH and a piece of fluted filter paper. Two-tenths cc. of an appropriate dilution of specific or control antibody was placed in the side arms. The latter were mixed after equilibration and the initial reading. Readings were ordinarily made every hour for 5 hours.

For the ultraviolet absorption measurements 2 cc. were removed from the well-shaken flasks. This was centrifugalized at high speed and washed 3 times with centrifugalization. The precipitated organisms were then hydrolyzed with 0.1 N NaOH, incubated overnight and ultraviolet absorption curves determined with the Beckman spectrophotometer—Model DU.*

RESULTS

Effect of Specific Antibody on O_2 Consumption of S. marcescens *(JH)*

Figure 1 shows the results of a typical experiment to test the effect of specific antibody on *S. marcescens* (JH). During the first 2 hours O_2 consumption is low and there is relatively little difference. In the third and ensuing 2 hours there is marked acceleration in O_2 uptake of the agglutinated bacteria in contact with antibody. By the end of the 5th hour too many bacteria are present to continue recordings as O_2 consumption is so rapid, unless, of course, the manometers are opened and reset.

Comparative Effect of Specific Antibody on O_2 Consumption of S. marcescens, *(Strains JH, SC, GC, and UM)*

In Table I are shown comparative effects of specific antibody on 4 strains of *S. marcescens*. It will be noted that different dilutions have been used since the growth activities are so variable. The differences in response to antibody are extreme. It should be pointed out that the specific antibody was prepared

*We are indebted to Miss Jean Fisher for these determinations.

in rabbits using the strain JH; however, strains SC, GC and UM are equally affected as far as O_2 consumption is concerned and agglutinated.

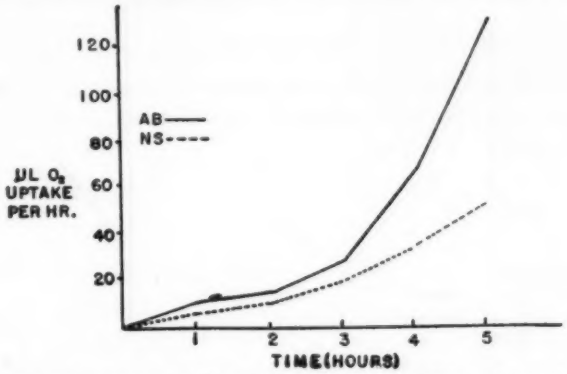

FIG. 1. Comparative curves of O_2 consumption in microliters of growing cultures of *S. marcescens* (strain JH). All flasks contain 3 cc. medium (trypticase soy broth) and .2 cc. 20% KOH in the center well. Flasks AB contain .2 cc. of a .2% solution of purified specific antibody, while flasks NS contain a .2% solution of antibody for *S. typhosa*. Manometers are read every hour for 5 hours.

TABLE I

Comparison of O_2 Consumption of 4 Strains of S. marcescens *with and without Antibody*

STRAINS AND DILUTION		4TH HOUR		5TH HOUR	
		O_2 Uptake	Ratio O_2 Uptake AB/NS	O_2 Uptake	Ratio O_2 Uptake AB/NS
SC.	AB	86	1.9	302	2.0
1–5000	NS	44		150	
JH.	AB	103	2.6	204	2.3
1–500	NS	39		89	
GC.	AB	64	3.05	135	5.0
1–100	NS	21		27	
UM.	AB	161	12.4	506	19.5
1–1500	NS	13		26	

The Relationship of O_2 Consumption to Growth of S. marcescens *(JH) in the Presence of Specific Antibody*

In our earlier experiments (20) the possibility had been entertained that the increased O_2 consumption encountered in the presence of specific antibody might be explained by an acceleration of growth. This possibility was further strengthened when growth was inhibited by appropriate amounts of strepto-

mycin. Figure 2 shows the results of another characteristic experiment studying O_2 consumption of *S. marcescens* (JH) in the presence of specific and non-specific antibody. In Figure 3 are shown ultraviolet absorption curves of the material

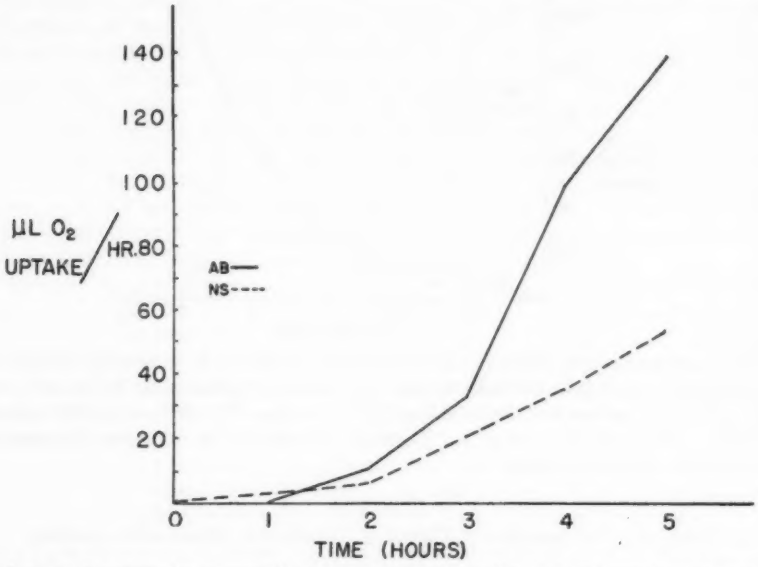

FIG. 2. Comparative curves of O_2 consumption in microliters of growing cultures of *S. marcescens*. Conditions same as defined in Figure 1.

TABLE II

Phosphorus and Nitrogen Concentrations of Centrifugalized Contents of Flasks with and without Antibody for S. marcescens*

STRAIN	P CONTENT IN MG. %		N CONTENT IN MG. %	
	AB	NS	AB	NS
MD	.84	.2	7.5	1.25
MD	.64	.18	6.2	1.2
JH	.32	.17	2.5	0.18
JH	.4	.2	1.2	0.4

* 2 cc. of the well shaken contents of duplicate flasks were centrifugalized (10,000 rpm), the sediments washed with saline 2× and digested with 0.1 N NaOH.

obtained from the flasks, processed as outlined above. It will be noted that the peaks of both curves occur at approximately 2600 Å; the curve for the material obtained from the reaction flasks containing antibody lies above that of the controls, presumably indicating more nucleic acid in the former and hence more organisms.

Table 2 shows the results of nitrogen and phosphorus determinations on similar material. Both of these substances are increased in the sediment from the antibody-containing flasks.

The Relationships of O_2 Consumption to Number of Organisms

It was thought desirable to obtain data dealing with the relationships o the hourly O_2 consumption, without antibody, of course, and number of or-

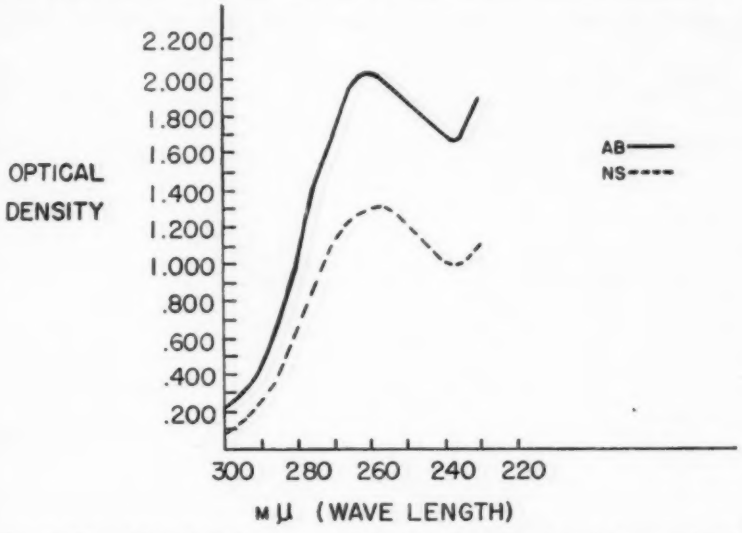

FIG. 3. Ultraviolet absorption curves, optical density plated against wave length for aliquots of contents of flasks whose O_2 consumptions are shown in Figure 2. Maxima are found at 2600 Å but optical density of material from flasks containing specific antibody is greater.

ganisms in order to be certain just where the lag phase ended and the logarithmic phase began under the conditions of our experiment with the organism *S. marcescens* (strain JH). This relationship is shown in Figure 4 where it will be noted that aside from slight deviation at the beginning the two curves are parallel. This would appear to indicate that O_2 consumption and growth are constant and equal, a relationship which has been shown to be present by others (17).

Effect of the Addition of Complement on O_2 Consumption of S. marcescens *in Presence of Specific Antibody*

Inasmuch as the *gamma* globulin fraction, i.e. antibody, contained no complement it was felt desirable to determine what effect, if any, complement might

have on the system. Guinea pig complement in excess of that used for routine complement fixation tests was added to flasks containing specific and non-specific antibody. There was no effect on the customary difference in O_2 consumption between the two.

A Comparison of the Effects of Specific and Non Specific Antibody on the O_2 Consumption of S. marcescens *for Prolonged Periods*

It was thought desirable to study a longer time interval than the usual 5 hour period in order to determine whether the observed marked difference

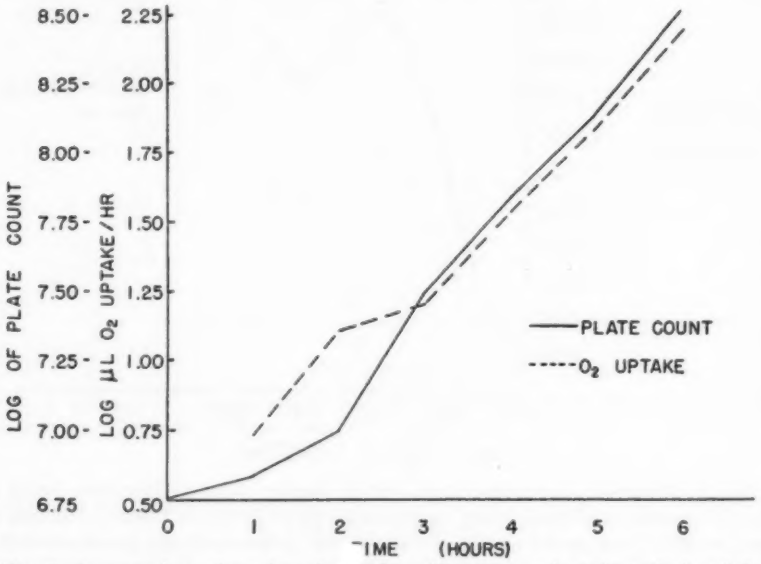

FIG. 4. A comparison of number of organisms (*S. marcescens*) at the end of each hour for 6 hours (as determined by plate counts) with the O_2 consumption at similar intervals. Except for some deviation at the beginning in the lag phase, the curves are parallel.

would be maintained. Because of the increasing numbers of organisms in both pairs of flasks the manometers had to be opened each hour and after the 6th hour O_2 consumption was so great that readings could be taken for only 15 minutes; such values were multiplied by 4. In addition, during the last 4 hours the flasks were gassed with O_2. As a further modification in technique, 40% KOH rather than the usual 10% was placed in the center well. Observations were made for 14 hours, that is, during and past the entire logarithmic phase. Values for O_2 consumption are shown graphically in Figure 5. The usual difference is seen up to the 11th hour; then, as the O_2 consumption in the antibody containing flasks, drops that in the control flasks takes a marked spurt to fall

in a similar fashion in the next hour. The cause for this phenomenon is not clear.

The Effect of Repeated Cultivation in Media Containing Specific Antibody on the Ensuing Organisms (S. marcescens)

The possibility that the strain of *S. marcescens*, JH, which has been used in most of these studies might undergo mutation with subsequent acceleration in

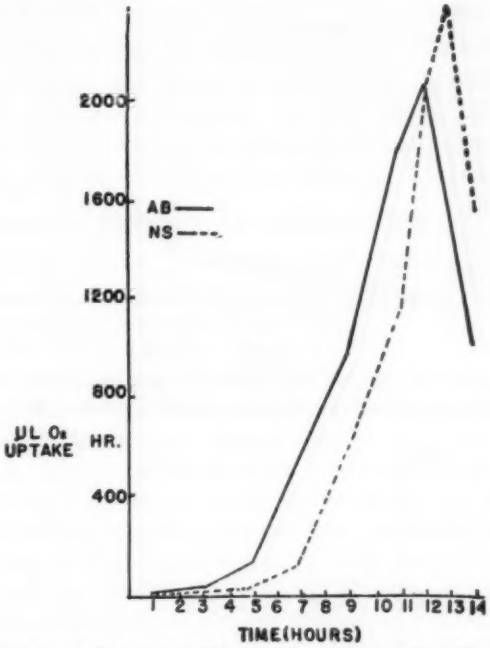

FIG. 5. A comparison of O_2 consumption of *S. marcescens* with (AB) and without (NS) antibody over a prolonged period. Conditions the same as those noted in Figure 1 except .2 cc. of 40% KOH were placed in the center well and antibody concentration, both specific and non-specific, was quadrupled.

its metabolism and growth when cultivated in contact with its specific antibody for even short periods prompted us to reculture repeatedly (10 times) in the presence of specific antibody. When compared with the stock culture no difference in O_2 consumption could be detected.

Effect of Specific Antibody on the Size of Colonies of S. marcescens *in Contact with Antibody*

It was thought desirable to determine what effect, if any, specific antibody might have on surface colonies. Here one would be dealing presumably with

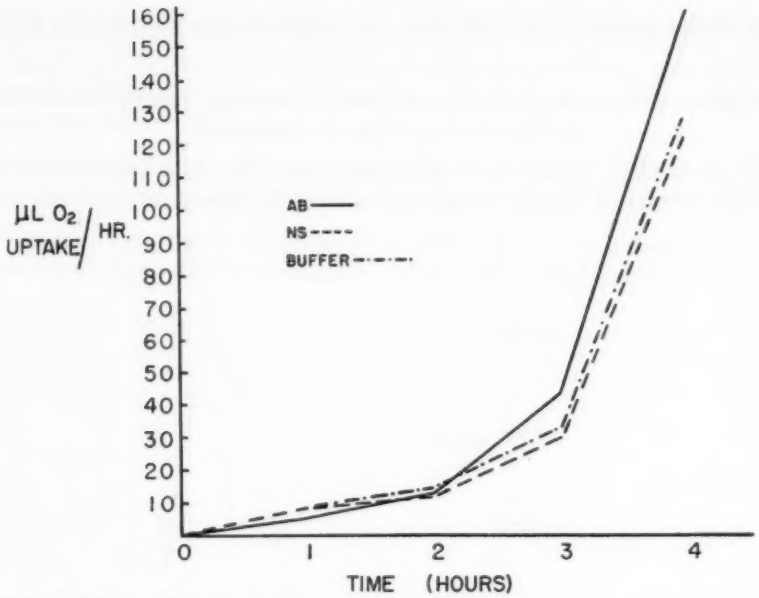

FIG. 6. Comparative O_2 consumption curves of a growing culture of *S. typhosa* with specific antibody (*S. typhosa*) and non-specific antibody (*S. marcescens*).

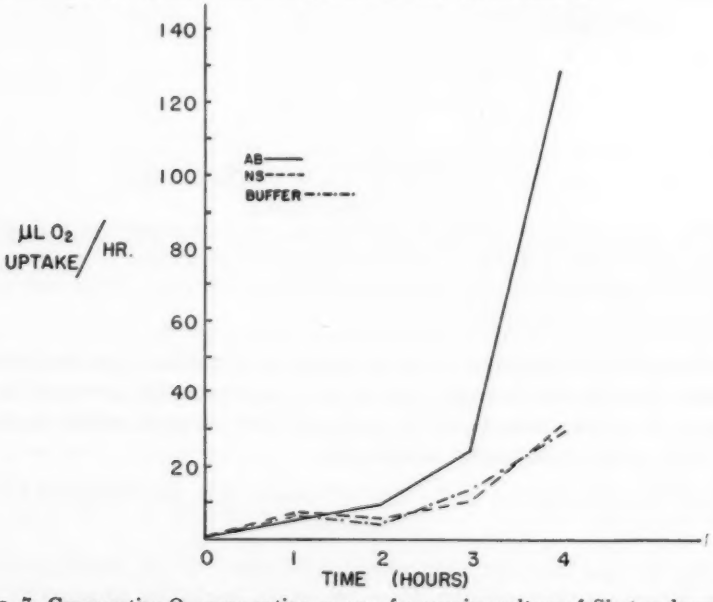

FIG. 7. Comparative O_2 consumption curves of a growing culture of *Sh. paradysenteriae* with specific antibody (*Sh. paradysenteriae*) and non-specific antibody (*S. macrescens*).

single organisms at the beginning, and the effect of agglutination would be ruled out.

Pour plates were made in extract agar using an appropriate dilution of organisms. After allowing the plates to solidify, one set was flooded with a small amount of specific antibody, the other set was similarly treated with a non-specific antibody. The excess antibody solutions were drained off and the plates incubated at room temperature for 96 hours. There was no difference in the size of the ensuing colonies.

Effect of Specific Antibody on O_2 Consumption of S. typhosa

In Figure 6 are shown the results of a typical experiment to study the effects of specific antibody on *S. typhosa* (H 901). Although a difference, i.e. an acceleration of O_2 consumption, is elicited by the presence of antibody, it is not particularly marked, for this strain at least.

Effect of Specific Antibody on O_2 Consumption of Sh. paradysenteriae

Figure 7 shows the results of a typical experiment to test the effects of specific antibody on *Sh. paradysenteriae* (F 16). A pattern similar to that which has been noted with the other organisms is found.

DISCUSSION

A number of studies on the effect of specific antibody on the O_2 consumption of various organisms have been reported: Wiesel, 1912 (5), *B. typhosum*; Verzár and Bögel, 1920 (9), *paratyphosus B*; Surányi and Pálóczy, 1930 (10), *pyocaneus*; Wohlfeil, 1933 (13), *B. typhi*, *B. paratyphi*, and *B. Schottmuller*; Zih, 1939 (14), *B. paratyphosus B* and *B. coli*; von Jenny and Vaczi, 1940 (15), *B. coli*; Kallós, 1941 (16), *B. paratyphosus B*; Olfeld, 1942 (17), *Flavobacterum* and *Pyocyaneus*; Sevag and Miller, 1948 (18), *E. typhosa*; and Harris, 1948 (19), various types of Salmonella. None of these observers noted any increase in O_2 consumption such as we have described. Most found no difference. Two groups, Surányi and Pálóczy (10) and von Jenny and Vaczi (15), noted some decrease which they attributed to the marked clumping of the organisms.

How are the contrary results we have obtained to be explained in the face of such an array of negative evidence? The answer is fairly clear. All of those who have studied this problem, ourselves included in earlier experiments, used old cultures (post-logarithmic phase), at least when techniques employed were adequately described. It was not until we turned to the study of organisms during their logarithmic phase of growth that any effect could be detected. This is the first time growing organisms have been employed in such experiments.

That specific antibody should cause an increased growth of organisms is truly surprising. At the present time we are not in a position to offer a definite explanation of this phenomenon, though several hypotheses can be suggested.

Perhaps the most likely one is based on the physical aggregation of the organisms. Several investigators have suggested and brought forth evidence that during the lag phase, bacteria liberate a substance of a stimulatory nature, probably a protein, into the surrounding medium. Malmgren and Heden (23, 24), have concluded from their experiments that "during the lag phase, the bacteria supply a substance necessary for growth to the surrounding medium and that the production of this substance is linked up with nulceotide metabolism." If such a material were liberated by agglutinated organisms in the flasks containing specific antibody it might be present locally in the aggregated mass in higher concentration that it would in the flask containing unagglutinated organisms. In the latter one would expect it to diffuse out into the medium. Proof for this hypothesis will necessitate the production of agglutination in the absence of specific antibody. We are attempting to achieve this at the present time and in addition by the use of synthetic media are attempting to inhibit the phenomenon of increased O_2 consumption in the presence of specific antibody.

Another possibility must be considered: that the union of antibody and bacterium in some way changes the surface of the latter, making it more permeable perhaps, so as to allow the transfer of metabolites of nutrients more readily and so increase metabolism and growth. Since we know little about the "osmotic barrier" (25) of bacteria, one cannot carry this hypothesis very far.

Since some workers studying the effect of specific antibody on O_2 consumption had noted that the addition of complement caused an increase (10), (15) or decrease (14) or no effect over the controls unless lysis occurs (18), the experiments dealing with the addition of complement referred to above were carried out. Our results with *growing* organisms indicate that complement has no effect on the action of specific antibody.

Since an acceleration in O_2 consumption and growth of the organisms has been observed, it would seem likely that we are not dealing with an anti-enzyme effect. Although specific antibody has been shown to be effective in inhibiting certain enzymatic activities of bacteria (5, 6, 7, 8, 11, 12), no evidence has been brought forward to indicate an inhibition in respiratory activity. Sevag (26) points out that this may be due to the large size of the antibody molecules since a combination of oxidative enzymes, doubtless situated within the cell, with antibody molecules deposited on the surface could not take place. Sevag (26) makes the suggestion that cell-free enzyme preparatons will have to be employed for immunization and enzyme-antibody studies.

SUMMARY

The effect of specific antibody prepared in rabbits on the oxygen consumption of 4 strains of *S. marcescens* and one each of *S. typhosa* and *Sh. para-*

dysenteriae has been investigated. It has been demonstrated that there is an increase in oxygen consumption of the agglutinated organisms and this increase is due to an increased growth of the bacteria. The underlying mechanism for this has not yet been elucidated.

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STUDIES OF THE EFFECT OF AUREOMYCIN ON THE PRODUCTION OF PENICILLINASE BY STAPHYLOCOCCI

CAROLINE A. CHANDLER AND ERITHA VON DER GOLTZ

The Departments of Preventive Medicine and Pediatrics, The Johns Hopkins University School of Medicine

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In a study on the resistance of staphylococci to penicillin conducted in 1950–51 (1), it was found that 52% of 50 pathogenic strains and 16 % of 25 respiratory strains were resistant to more than one microgram of penicillin on primary isolation. All but one of the resistant strains produced penicillinase whereas none of the sensitive strains produced this enzyme. It was further found that repeated exposure of a highly penicillin resistant strain to aureomycin resulted in permanent loss of the strain's ability to produce penicillinase.

The present study was undertaken to find out what effect, if any, *limited* exposure of both penicillin resistant and penicillin* sensitive strains to another antibiotic (aureomycin)* might be. Accordingly, out of the 75 strains of staphylococci previously studied, 27 were selected, 6 of which were moderately to highly resistant, 6 were highly resistant, and 15 were sensitive to penicillin. After initial testing for penicillinase production and sensitivity to penicillin and aureomycin, each strain was arbitrarily exposed to ten transfers in aureomycin broth and then to ten transfers in plain heart infusion broth. The changes in penicillin sensitivity and penicillinase production following such exposure are given under RESULTS.

MATERIALS AND METHODS

The strains of staphylococci isolated from pathogenic processes were obtained in pure culture from the bacteriology laboratory of the Harriet Lane Home and the general biological laboratory of the Johns Hopkins Hospital last year. Strains Edwards, Mason, Stokes, and Brown were isolated from nose and throat cultures of healthy individuals who sought employment in the Johns Hopkins Hospital. Of the twenty-three pathogenic strains, 20 (86.9%) were hemolytic *Staphylococcus aureus* and 3 (13.1%) were *Staphylococcus albus*. Of the four strains from the respiratory tract, 2 were hemolytic *Staphylococcus aureus* and 2 were *Staphylococcus albus*. All strains of *Staphylococcus aureus* from whatever source were coagulase positive while all strains of *Staphylococcus albus* were coagulase negative.

The susceptibility of the strains to penicillin was determined by the serial twofold dilution method employed by (2) this laboratory. The size of the inoculum routinely employed was a 10^{-4} dilution of the test strain. End points were recorded at the end of twenty-four and seventy-two hours incubation of the cultures and drug dilutions.

The production of penicillinase was determined by the disc method. Plates containing

* Crystalline penicillin G was supplied by E. R. Squibb & Sons; Aureomycin was supplied by Lederle Laboratories, American Cyanamid Co.

15 cc. of heart infusion broth agar were seeded with 1 cc. of a 10^{-4} dilution of a spore suspension of *B. subtilis*. The strain selected was known to be highly sensitive to penicillin and it did not produce penicillinase. After thorough wetting of the plates the excess suspension was removed with a pipette and the plates were dried in the incubator for twenty minutes. Sterile filter paper discs, 12.7 mm. in diameter, were then saturated with broth containing (a) penicillin in three standard concentrations—2, 10, and 100 micrograms per cc. and (b) penicillin to which had been added a sufficient amount of an 18 hr. broth culture of the test organism to give a final concentration of 2, 10, and 100 micrograms per cc. On the surface of each plate, prepared in the manner described above, two discs saturated with 2 test strains plus penicillin were applied together with one control disc saturated with penicillin concentrations of 2, 10, and 100 micrograms per cc. The plates were incubated for eighteen hours, after which the amounts of penicillinase produced by the test strains were estimated by measuring the zones of inhibition surrounding the test discs as compared with the zone surrounding the control disc. By this method, a strain which produced a large amount of penicillinase would show no zones of inhibition in all three concentrations; a strain producing no penicillinase would exhibit zones of inhibition comparable to the controls in all three concentrations; a moderate penicillinase producer would show no zones of inhibition at concentrations of 2 and 10 micrograms per cc., but a zone comparable with the control at 100 micrograms per cc.

In the previous study (1950–51), an effort was made to find out if the production of penicillinase by a given strain could be augmented by repeated transfers in broth containing penicillin or decreased by repeated transfers in broth containing another antibiotic. With this in mind, Strain 8, a penicillin resistant strain, was tested for its susceptibility to aureomycin. It was then transferred daily in broth containing aureomycin in increasing concentrations for thirteen transfers and, as a control, the same strain was transferred daily in plain broth. The strain evolved from the transfer in aureomycin no longer produced penicillinase, nor did the original properties return upon three months refrigeration on heart infusion broth agar and subsequent transfer in heart infusion broth. However, similar work was done in the fall of 1951 on penicillin resistant and penicillinase producing Strain 3, and it was discovered that though the strain lost its ability to produce penicillinase during its transfer in aureomycin broth, it regained it during transfer in heart infusion broth. The following experiment was devised to discover the reactions of 27 of the strains included in the previous study to this type of treatment.

First, each strain's penicillin sensitivity, aureomycin sensitivity and ability to produce penicillinase was tested. Then, the strain was simultaneously transferred for ten days in heart infusion broth and in increasing concentrations of aureomycin.* This resulted in Strain A and Strain DT (daily transfer). Each resulting strain was tested for its penicillin sensitivity and ability to produce penicillinase. Then both strains were transferred ten times in heart infusion broth and retested for their penicillin sensitivity, aureomycin sensitivity and ability to produce penicillinase.

RESULTS

Of the 27 strains of staphylococci tested, 6, or 22%, (strains 3, 4, 5, 6, 9, 40), resistant or moderately resistant to penicillin, lost their ability to produce

* In the previous study the inoculum had been 0.1 cc. of a 24 hr. growth of bacteria in the aureomycin broth of the preceding test. In this study, the serial two-fold dilution method was set up, in which the inoculum used was a 10^{-4} dilution of the contents of the last tube showing growth from the preceding two-fold dilution.

TABLE I

TABLE I

Comparison of the in Vitro *Sensitivity to Penicillin and the Production of Penicillinase by Strains of Staphylococci Before and After Exposure to Aureomycin and After Subsequent Exposure to Heart Infusion Broth*

STRAIN	BEFORE AUREOMYCIN					AFTER 10 TRANSFERS IN AUREOMYCIN					AFTER 10 TRANSFERS IN HIB				
	Peni sensitivity (Serial 2-fold dilution γ/cc)		P'ase production (Zone of inhibition—mm.)			Peni sensitivity (Serial 2-fold dilution γ/cc)		P'ase production (Zone of inhibition—mm.)			Peni sensitivity (Serial 2-fold dilution γ/cc)		P'ase production (Zone of inhibition—mm.)		
	18 hr	72 hr.	2γ	10γ	100γ	18 hr.	72 hr.	2γ	10γ	100γ	18 hr.	72 hr.	2γ	10γ	100γ
3 (original)	6.25	>100	—	—	—										
3 (DT)						3.12	>200.	—	—	—	.78	25	—	—	—
3 (Aureo)						.78	6.25	7.3	9.8	17.6	3.12	12.5	—	—	—
4 (original)	.78	25	—	—	—										
4 (DT)						12.5	12.5	—	—	—	50	50	—	—	—
4 Aureo)						.78	12.5	7.3	9.8	10.8	12.5	12.5	—	—	—
8 (original)	50	>100	—	—	26.3										
8 (DT)						>100	>100	—	—	—	>100	>100	—	—	—
8 (Aureo)						>100	>100	—	—	27.3	>100	>100	—	—	—
27 (original)	6.25	>100	—	—	24.3										
27 (DT)						100	>100	—	—	—	6.25	50	—	—	—
27 (Aureo)						50	>100	—	—	—	12.5	25	—	—	—
25 (original)	.156	.312	—	17.3	30.3										
25 (DT)						.312	>10	—	10.3	25.3	<.156	1.25	—	—	19.3
25 (Aureo)						.312	>10	8.8	15.8	27.3	<.156	1.25	—	—	15.3
Ma (original)	<.78	<.78	15.3	21.3	25.3										
Ma (DT)						<.078	.078	16.3	19.3	30.3	<.078	<.078	16.8	25.3	31.3
Ma (Aureo)						<.078	.156	18.3	23.3	28.3	<.078	<.078	16.8	25.3	31.3

DT = 10th transfer in HIB.
Aureo = 10th transfer in aureomycin.
— = no measurable zone of inhibition i.e., abundant penicillinase.

penicillinase during transfer in aureomycin and then regained it during transfer in heart infusion broth. Six, or 22% (strains 8, 27, 28, 30, 32, 41), highly resistant to penicillin, underwent no apparent change during transfer in aureomycin. Fifteen, or 56% (strains 17, 18, 23, 24, 25, 26, 33, 42, 44, 46, 50, Ed, Bro, Ma, St), sensitive, non-penicillinase producing strains, underwent no apparent change during transfer in aureomycin broth. Strains 25, 26, 33, and 34 actually seemed to acquire penicillinase producing capacity after ten transfers in heart infusion broth, but since the controls exhibited the same behavior it is difficult to interpret the possible significance of this observation. Two representative strains from each of the above three groups were selected for presentation in Table I.

All strains had been refrigerated for three months on slants of heart infusion broth agar. Before testing, each "original" strain was transferred twice in heart infusion broth. Frequently the original's properties were not the same as those of the daily transfer, (DT), ten transfers later. This suggests that the growth of the test strain was not logarithmic at 24 hours by the second transfer after three months refrigeration as it probably was by the tenth.

DISCUSSION

In a previous study (1), it was found that the capacity of a penicillin resistant strain of *Staphylococcus aureus* to produce penicillinase could be lost by repeated exposure to another antibiotic (aureomycin) and that this loss was irreversible. In the case of the resistant organism, Strain 8, this loss of ability to produce penicillinase was accompanied by a shift in penicillin resistance from high degree to low degree, but re-acquisition of resistance did not lead to re-acquisition of the capacity to produce penicillinase.

In the present study, 27 strains of staphylococci from the previous study were arbitrarily exposed to ten transfers in aureomycin broth and then to ten transfers in heart infusion broth. Six strains of moderate to high resistance lost their ability to produce penicillinase after exposure to aureomycin but regained it after subsequent transfer in heart infusion broth. This transient loss was accompanied by a slight shift in penicillin resistance towards the more sensitive range but this shift, too, was transient. Six highly resistant strains underwent no apparent change in either ability to produce penicillinase or resistance to penicillin following ten transfers in aureomycin. A word is necessary to explain the behavior of Strain 8 which is included in this highly resistant group and which in the previous study had permanently lost its penicillinase producing capacity after exposure to aureomycin. One factor is the number of transfers in aureomycin, the present study having arbitrarily set the number at ten and the previous one having had as its goal the length of time necessary to lose penicillinase producing capacity regardless of the number of transfers in

aureomycin. The other factor of perhaps even greater importance was the difference in the size of the inocula employed in the two studies. Relatively large inocula were used in the former study as compared with the smaller inocula used throughout these present observations and this factor alone could account for the difference in the two studies with regard to this one strain.

Fifteen sensitive, non-penicillase producing strains were unaffected by ten transfers in aureomycin broth.

To summarize these observations it would seem apparent that exposure of penicillin resistant or moderately resistant strains of staphylococci to another antibiotic may result in: (1) permanent loss of ability to produce penicillinase; or (2) transient loss of penicillinase producing capacity with subsequent return following transfer in media free of antibiotic; or (3) neither gain nor loss in ability to produce penicillinase. Two factors which would appear to be determining ones as to outcome with any given resistant strain are the number of transfers in the other antibiotic and the size of the inocula employed in transfer.

Sensitive, non-penicillinase producing strains of staphylococci are unaffected by exposure to another antibiotic.

SUMMARY

1. Of the 27 strains of staphylococci included in this study, 22% which were of moderate to high resistance to penicillin, lost their ability to produce penicillinase during limited exposure to another antibiotic (aureomycin) but regained it following subsequent transfer in media free of antibiotic.

2. Of the 27 strains, 22% which were highly resistant to penicillin, retained their full capacity to produce penicillinase in spite of limited exposure to another antibiotic.

3. Of the 27 strains, 56% which were penicillin sensitive, non-penicillinase producers, underwent no change following exposure to another antibiotic.

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THE PREVALENCE OF RICKETS AT AUTOPSY DURING THE FIRST TWO YEARS OF AGE*

RICHARD H. FOLLIS, JR., EDWARDS A. PARK AND DEBORAH JACKSON

The Departments of Pediatrics and Pathology, The Johns Hopkins University School of Medicine and the Harriet Lane Home of The Johns Hopkins Hospital

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INTRODUCTION

In 1943 we (1) reported on the prevalence of rickets in a group of 230 children, two to fourteen years of age, whose skeletal tissues were studied at autopsy. The overall incidence of rickets in this series of cases was found to be 46.5%. The present communication, which is a continuation of our previous study, takes up the occurrence of rickets from birth through the first two years of age. The time period covered by both is the same, i.e. from 1926 through 1942.

As we have pointed out before (1) the only report on the autopsy prevalence of rickets is that of Schmorl (2). We shall comment on the comparison of Schmorl's and our findings more fully below.

Because rickets is difficult to recognize both clinically and roentgenographically in its early stages, post mortem examination offers the most delicate and precise method with which to gain some idea of its prevalence in a group of individuals dying of a variety of causes. The purpose of the present communication is to indicate the prevalence of rickets in a series of 1303 children, dying during the first 2 years of age. In addition we shall call attention to certain factors which have some bearing on the prevalence of the disease in this age group.

Rickets is characterized by a failure of inorganic materials, particularly calcium and phosphorus, to be deposited in the organic matrices of cartilage and bone (osteoid). The great majority of instances of rickets results from some derangement in the humoral concentrations of calcium and/or phosphorus, whether due to an inadequate dietary supply or poor calcium (and phosphorus) absorption as a result of vitamin D deficiency. The decrease in the normal serum concentrations of calcium or phosphorus leads to defects in the calcification of cartilage and bone matrix; as a result the framework of cartilage matrix and trabeculae are covered to varying degrees with borders of uncalcified bone matrix or osteoid. Endogenous rickets, of course, may be encountered as a result of chronic renal disease, celiac disease, the de Toni-Fanconi syndrome which may be associated with cystinosis, etc. We shall not go into a detailed

* Aided by grants from the Children's Bureau, U. S. Department of Labor and Mead Johnson and Company.

description of the morphological aspects of rickets at this time; this, together with data on the x-ray changes, will be taken up in other communications.

However, in order that the reader will be familiar with the terms which will be introduced we shall have to allude briefly to our criteria of rickets, examples of which are illustrated in Figures 1–14. Rickets must be considered, like any other disease, with respect to its severity and duration. To this end we have attempted to set up a more or less conventional classification as to whether the lesions were to be considered slight, moderate or severe and whether the disease was of short duration, that is acute (14 days or less) or longer, i.e. subacute or chronic. For the estimation of duration we relied chiefly on the extent to which endochondral ossification was affected. If growth is preserved, given time enough, severe and moderate rickets will leave characteristic patterns at the ends of the bone consisting of typically deformed proliferative cartilage and trabeculae. If this rachitic zone was broad, for example 1 cm. or more at the anterior end of one of the middle ribs, we were able to infer that the disease had been present for some time and could classify the case as chronic. At the opposite extreme, if the rachitic disturbance consisted merely in defects in calcification of the provisional zone of the cartilage with or without beginning invasion and there was no rachitic zone at all, meaning that not enough time had elapsed since the beginning of the disease for the end of the shaft to develop the defects in pattern, we classified the case as acute, except in numerous cases of slight rickets in which we could not exclude the possibility that the disease might have been subacute or chronic, as will be explained later. The classification of subacute was made when evidence for the disease was present but was not extensive enough to indicate long duration. We hasten to point out that the division of our cases in terms of duration was subject to great difficulties of interpretation and some error on the side of omission. As is well known, the rate of growth at the ends of the various long bones of the body varies tremendously. It is most rapid in the middle ribs, next at the lower end of the femur and slowest at the upper ends of the radius and ulna, particularly the latter. Severe chronic rickets in one of the middle ribs might be evidenced by a zone a centimeter in length; whereas at the upper end of the ulna the lesion would be limited to the cartilage-shaft junction (Figures 13 and 14). From the examination of the upper end of the ulna one might infer that the rickets had been of extremely short duration and from the rib that it had been chronic. We easily got round the difficulty of the variation of the growth rate in the two ends of the long bones because in almost every case one of the middle ribs was available for study. It was on the findings in one of the middle ribs that we depended chiefly for our estimation not only of the duration of the disease but also of its degree. But a difficulty which we could not circumvent was that the general growth rate must have been subject to great variation in our series of children, since they were not only of different ages but

died from a whole variety of diseases of different severities and durations which doubtless had different effects on growth. There was no means by which we could estimate the extent to which the growth rate had been affected by the disease; nor are there accurate data concerning the increments of growth at the different bone ends in the different months of infancy. In many cases there was evidence that growth had been subject to checks; in some it had obviously been greatly reduced and in a large number, in particular the subjects of acute, violent infections like dysentery, growth had virtually ceased. In general, however, it was remarkable to see the evidence afforded by the rachitic lesions in the bones that growth had persisted in spite of prolonged serious illness. We were aided in other ways in making judgments of the duration of the disease. For example, if the baby was young enough, the disease was obviously acute. In some cases histological sections of the upper ends of the radius and ulna and in a considerably larger number x-ray films of those bone ends were available. If the rickets could be well established by either method at the upper ends of the radius and ulna, the disease was obviously chronic. We could tell that the rickets was chronic, also, in cases in which the cartilage had accumulated in large mass at the end of the shaft and the invading vessels, those entering from the cartilage side, the cartilage canals, and those ascending from the shaft side had time enough to become large bush-like structures. At times we were aided in estimating that the duration of the disease had been long because of the wideness of the osteoid borders of the trabeculae. Extremely broad borders indicated chronicity. But the development of the osteoid borders was subject to great variation and in a good many cases in which the rachitic lesion at the end of the bone was extensive, the osteoid borders in the shaft were not particularly broad and in some were almost non-existent. We were often aided in the recognition that the rickets should be classified as subacute because the vessels entering the cartilage from the shaft had succeeded in penetrating too far for acute rickets. In yet other cases we could see that the disease must have been at least subacute because of the depth which the proliferative cartilage had attained and also because of compression deformities of the cartilage at the shaft junction.

We encountered equal difficulties in estimating the degree of the rickets. Our chief criterion for estimating the degree was the extent to which the deposition of inorganic material appeared to have been affected at the cartilage-shaft junction. We knew, of course, that the rickets was severe when lime salt deposition in the cartilage was entirely suspended. But this state of complete rickets was encountered only occasionally and then only in cases in which the disease was acute or subacute. In almost all instances of chronic rickets, scattered deposits of inorganic salts which from their pattern could be identified as having occurred in cartilage matrix could be found somewhere in the rachitic

area. Balance studies in rickets in the human being have repeatedly shown that negative balances almost never occur, i.e. that some deposition of calcium, phosphate and carbonate must be occurring somewhere in the skeleton even when the rachitic state is most severe; it is noteworthy in our series that in no case in which the rickets had evidently been in progress for a long time were localized inorganic depositions entirely wanting. If the deposition of the lime salts in the middle rib was severely inhibited so that only a few scattered depositions could be found on microscopic examination, we called the rickets severe. On the other hand, if the provisional zone of calcification of the cartilage showed normal calcification everywhere except in a few isolated points, we classified the case as slight. We classified cases as moderate when deposition of inorganic materials was considerable but irregular with areas of deposit alternating or intermingled with areas which had been completely spared. In estimating the degree of rickets we did not get much help from the osteoid on account of its variability resulting probably from general nutritional conditions rather than from the effects of the rickets itself. We did of course obtain help from the examination of osteoid in the identification of healing and healed rickets.

Our classification gave rise to nine combinations but two of these proved impractical. We were able in some children to be certain that slight rickets was acute, but in many cases it was impossible to be sure that it might not have been subacute or chronic. In other words, we could not recognize subacute or chronic rickets which had remained slight. The reason for this was that rickets, when slightly developed at the cartilage-shaft junction, that is when limited to a few spotty defects in calcification of the provisional zone, does not leave behind it any record in the form of deformed trabeculae in the shaft distinct enough for recognition. This finding of ours is important in the evaluation of the only study of the incidence of rickets similar to ours, namely that of Schmorl which will be discussed below.

Subacute moderate rickets was our dumping ground. Its cases exhibited a wide latitude both in duration and degree, for, if we could not classify a case as definitely chronic and severe or slight, we put it in the subacute category.

When healing occurred in rickets which had reached either the subacute or chronic stage, it was easy to recognize from the presence of a telltale sheet of inorganic materials, always incomplete, stretching across the cartilage at the level of the most recently differentiated cells. It is important to emphasize that if this sheet occupied the level of the provisional zone, we knew that healing was occurring at the time of death. In many cases, however, such sheets lay in the cartilage on the shaft side of the provisional zone of calcification. When this occurred it was possible to infer that a healing episode had occurred but the rickets had broken out again subsequently and was severe at the time

of death. We were aided also in recognizing that active healing was in progress when the osteoid borders showed calcification along their junctions with the prerachitic calcified bone.

We were able to recognize healed rickets when we found that calcium-phosphate deposition in the cartilage had become fully resumed and the trabeculae normally calcified. In many cases of healed rickets indubitable evidence that it had been moderate or severe lay in the scars left by the disease which consisted not only of deformed trabeculae but often in addition of islands or strands of degenerated cartilage which had not yet been converted into bone and removed.

We have called attention to various difficulties encountered in estimating the duration and the degree of the rickets. But perhaps the greatest difficulty of all in classifying our cases lay in the fact that in many instances the disease at the time of death was not stationary but was rapidly changing its severity. For example, the study of the rachitic area might show that the disease had been of moderate severity but at the time of death had become so severe that deposition of calcium-phosphate had stopped altogether. In other instances the movement of the disease had occurred in the opposite direction, for example, a rickets which had been clearly severe or moderate had begun to show signs of healing. In still other cases there was evidence that the disease had fluctuated to and fro.

In experimental rickets in the rat the problem of estimating the degree of the disease is simple since the animals are standardized in regard to age or weight and controlled. In the case of our children, as already pointed out, no standardization was possible because the ages were different and because of disease factors. Our criteria of rickets could not be based on fixed measuring scales of any kind. In consequence our judgments how to classify our cases were entirely subjective and resolved themselves into decisions based on whatever evidences the individual cases offered in the light of our experience. It is obvious that our classification is subject to great error and is of the roughest sort but we believe that it will serve the purpose of giving correct general ideas adequate to meet the clinical requirements.

The material upon which this study is based is derived from a series of consecutive autopsies on 1303 white and negro children ranging in age from the first day to the end of the second year. One or more ribs, usually the middle group, were examined in virtually every instance. When available the epiphyseal portions of one or more long bones were also studied. We have presented the methods used in the preparation of these sections in another place (1). In order to facilitate analysis of the data, punch cards of the McBee Keysort type were utilized.

Some mention should be made of the racial distribution of the cases studied.

In Baltimore the negro population comprises about 20% of the total. In a given year discharges of negro children under 2 years of age average about 50% of the total. Another factor which produces an increase in the negro percentage in our series is that the percentage of autopsy permissions is higher in this group. In a 5 year period, 1937–42, the autopsy percentage was 80% in the negro and 65% in the white group.*

THE PREVALENCE OF RICKETS BY AGE

In Table I is presented the prevalence of rickets by age. Two cases were detected during the first 2 weeks of life, at 12 and 14 days. Both of these were

TABLE I

Prevalence and Severity of Rickets by Age

AGE	NO. SUBJECTS EXAMINED	NO. WITH RICKETS	PER CENT WITH RICKETS	PER CENT SLIGHT	PER CENT MODERATE	PER CENT SEVERE
0–15 days	360	2	—	—	—	—
16–30 days	90	55	61.2	36	49	15
1 mo.	99	48	48.5	21	67	12
2 mo.	90	69	75.9	9	48	43
3 mo.	69	57	82.7	19	63	18
4 mo.	79	62	78.5	34	29	37
5 mo.	73	56	76.8	30	52	18
6 mo.	53	44	83.0	21	52	27
7 mo.	54	49	90.7	25	47	28
8 mo.	40	30	75.0	23	63	14
9 mo.	38	30	79.0	27	43	30
10 mo.	41	26	63.5	30	35	35
11 mo.	40	24	60.0	21	37	42
12–23 mo.	177	113	63.8	36	30	34

classified as "acute slight." We have not observed a case of congenital rickets in a series of 162 stillborn infants which have been reviewed specifically for that purpose. It will be noted that from the first month on the prevalence rises and reaches a peak of 90.7% during the seventh month, falling thereafter, so that during the second year the prevalence for the 177 subjects studied was 63.3%. In order to have some idea as to the degree of severity of the rickets we have also included in Table I a breakdown of the cases in each age period as to whether a given instance was considered to be of slight, moderate or severe degree. It will be noted that those deemed of slight degree comprised on the average one quarter to one third of the total. In other words rickets was of moderate to severe intensity in three quarters to two thirds of all the cases.

*We are indebted to Dr. R. V. Rider of the Statistics Department, Johns Hopkins Hospital, for these data.

THE PREVALENCE OF RICKETS WITH REGARD TO COLOR

In Table II are presented data pertaining to the prevalence of rickets in white and negro children in the same age groupings used in Table I. In addition the percentages of cases designated as moderate and severe have been included. On the whole the percentage of cases of rickets in the white group is a little lower than that in the negro. The prevalence from the first month through the 11th month is 67.7% for the white and 71.9% for the negro children. There is a tendency for the disease to be more severe in the negro in that

TABLE II

Prevalence and Severity of Rickets by Color

AGE	NO. WHITE SUBJECTS	NO. WITH RICKETS	PER CENT WITH RICKETS	PER CENT MODERATE AND SEVERE RICKETS IN RACHITIC GROUP	NO. NEGRO SUBJECTS	NO. WITH RICKETS	PER CENT WITH RICKETS	PER CENT MODERATE AND SEVERE RICKETS IN RACHITIC GROUP
0–15 days	174				186			
16–30 days	41	25	60.9	61.0	49	30	61.4	70.0
1 mo.	48	19	39.5	68.5	51	29	56.9	86.2
2 mo.	34	21	61.8	90.5	56	48	85.8	91.7
3 mo.	31	25	80.5	72.0	38	32	84.3	87.5
4 mo.	26	22	84.6	41.0	50	40	75.6	80.0
5 mo.	25	17	68.0	70.5	48	39	81.3	69.3
6 mo.	19	15	79.0	60.0	39	29	85.3	89.7
7 mo.	22	19	86.4	63.0	32	30	93.8	83.3
8 mo.	18	13	72.2	77.0	22	17	77.3	76.4
9 mo.	12	10	83.3	70.0	26	20	22.0	75.0
10 mo.	16	11	68.9	54.5	25	15	60.0	80.0
11 mo.	12	6	50.0	33.3	28	18	64.3	94.3
12–23 mo.	68	31	45.5	45.1	109	82	75.2	69.6

the percentage of cases rated moderate and severe is greater in these children than in the white. Furthermore, if one takes into consideration only the cases designated as severe, of the 178 rachitic white children, aged 1 through 11 months, there were 21 or 11.8%, while in the 317 negro children autopsied at the same age, there were 115, or 36.3%.

Similarly, when the children dying in the second year are considered, the prevalence and severity of the rickets are greater in the negro than in the white group. The total prevalence is 45.5% for the white in contrast to 72.2% in the negro. As in the previous group, if one considers only those designated as moderate or severe the racial difference is just as striking, white, 45.1%, versus negro, 69.6%. So too, there were only 4 out of 68 white children having severe rickets (5.9%) while of the 109 negro children in this age group 34 or 31.2% were classified as severe.

THE RELATIONSHIP OF PREMATURITY TO THE PREVALENCE OF RICKETS

In Table III are presented data dealing with the influence of prematurity on the prevalence of rickets after the first month of life to the end of the 6th month. After this period the number of premature babies falls too low to be of significance. It will be further noted that only those cases designated as moderate or severe have been included. In the entire group the prevalence of moderate and severe rickets is not significantly higher in the prematures than in the full term infants, being 60% for the former and 56% for the latter children.

TABLE III

Relationship of Prematurity to Prevalence of Rickets in White and Negro Infants

AGE IN MONTHS	PREMATURE							FULL TERM						
	Total cases	Total White	Total Negro	Moderate and severe rickets				Total cases	Total White	Total Negro	Moderate and severe rickets			
				White		Negro					White		Negro	
				No.	%	No.	%				No.	%	No.	%
1	40	15	25	4	29.0	14	56.0	59	33	26	9	27	11	42.5
2	31	7	24	3		21	87.5	59	27	32	15	56	23	72
3	16	6	10	2		7		53	25	28	16	64	21	75
4	17	4	13	2		7		62	22	40	7		25	62.5
5	9	3	6	2		4		64	22	42	10		23	55
6	6	2	4	2		3		47	17	30	7		23	77
Totals	119	37	82	15	40.5	56	68.4	344	146	198	64	43.8	126	63.8

TABLE IV

The Seasonal Incidence of Rickets During the Second Year

SEASON	PERCENTAGE OF RICKETS
Winter (Dec.–Feb.)	36.4
Spring (March–May)	44.8
Summer (June–Aug.)	38.7
Fall (Sept.–Nov.)	32.5

THE RELATION OF SEASON TO THE PREVALENCE OF RICKETS

In Table IV are grouped by season the percentages of instances of subacute and chronic, moderate and severe rickets which occurred during the second year. Since no striking variations were found in the prevalence of rickets in this age group, the first year was not similarly investigated.

DISCUSSION

In this series of 1303 infants coming to autopsy from birth through the second year rickets began to manifest itself toward the end of the second week, in-

creasing in incidence, during the next two weeks. The peak incidence was reached during the middle portion of the first year after which the prevalence decreased so that during the second year the overall prevalence was 63.8%. It is of interest to note that this coincides with the falling rate which was encountered in a group of 230 older children which have been reported previously (1); during the third and fourth years the prevalence of rickets in this group was 57% and 50% respectively.

The only statistics of a kind similar to ours are those of Schmorl (2) who compiled his data from consecutive autopsies on children aged from 1 month to 4 years in Dresden, Germany, between the years 1901 and 1908. It is possible from Schmorl's tables to separate the data into periods from 1 month to the beginning of the third year so that they may be brought into comparison with ours. Before doing this, however, it is necessary to make clear differences in Schmorl's approach to the problem. Schmorl divided his cases into "beginning," "florid," "healing" and "healed" rickets. "Beginning" rickets occurred when the disease was demonstrable microscopically but did not show macroscopically or else was not evident enough on examination in the gross to make its presence certain. Rickets was "florid" when the disease was recognizable macroscopically, but healing was excluded by microscopic examination. "Healing" rickets was characterized by the presence of newly deposited inorganic material in the provisional zone of the proliferative cartilage, as identified by microscopic examination. "Healed" rickets was recognized when either characteristic rachitic gross deformities in the contour of the bones or rachitic deformities of the finer structure as revealed by the microscope were present but the osteoid did not exceed normal thickness and the cartilage failed to show fresh evidences of rickets, though it might still show still deformity. The second difference in Schmorl's approach to the problem was that as the result of preliminary studies he assumed rickets did not occur in the first month of life and, therefore, he stopped examining infants for rickets during the first month so that this period was left out altogether.

Schmorl found an overall incidence of rickets of 94.1 per cent in the 287 children whom he examined in the second to the 24th month. Schmorl's data are summarized in Table V. It will be seen that rickets began in the second month and from the second to the sixth month "beginning" cases prevailed and were common from the 7th to the 9th month. From the 10th month on "beginning" rickets gradually decreased although cases were encountered to the end of the second year. "Florid" rickets was rare to the 6th month but became frequent from the 7th month on, dominated from the 7th to the 9th month and reached its maximum at the end of the 1st year. From the 13th month on it decreased. "Healing" rickets appeared in the 2nd quarter of the 1st year and in the last half of the 2nd year rose to 36.6 per cent. "Healed" rickets was not encountered

until the 2nd year. The cases increased from a minimum of 1 to 2 per cent in the 1st half of the 2nd year to 23 per cent in the last half.

Comparisons of Schmorl's results with ours are difficult for a number of reasons, one of which is that his criteria of rickets were different from ours. He depended largely on examination of the bones in the gross. We found gross examination as a means of detecting rickets most unreliable, when checked by microscopic examination, except when the disease was far advanced. We could not recognize "beginning" rickets with enough certainty to make a separate classification of it for the following reasons: "Beginning" rickets is slight rickets. Slight rickets, as already pointed out, does not leave behind it any mark in the shaft, consisting in deformed trabeculae. Although often we could

TABLE V

Prevalence and Type of Rickets in Schmorl's Series from 2 to 24 Months of Age, Dresden, 1901–1908 (2)

AGE IN MONTHS	TOTAL SUBJECTS	NUMBER WITH RICKETS		TYPES OF RICKETS							
				Beginning		Florid		Healing		Healed	
		No.	%	No.	%	No.	%	No.	%	No.	%
2–3	33	20	60.0	16	80	4	20	—	—	—	—
4–6	34	33	97.0	19	57.6	7	21.2	7	21.2	—	—
7–9	53	50	94.0	17	34.0	23	46.0	10	20.0	—	—
10–12	75	73	97.3	7	9.5	45	61.7	20	27.4	1	1.4
13–18	59	58	98.3	4	6.9	32	55.2	15	25.8	7	2.1
19–24	33	30	90.9	2	6.6	10	33.4	11	36.6	7	23.4
Totals	287	264	94.1	65	24.6	121	45.8	63	23.9	15	5.7

be sure particularly from the age that slight rickets was "beginning" rickets, in many cases we could not be certain that slight rickets was not subacute or even chronic. All clinicians are aware that rickets in slight as well as severe form may be present for many months. We think that Schmorl's designation of "beginning" rickets should be changed to slight rickets, a classification which does not make any commitment as regards duration. Schmorl's "florid" rickets should correspond to our chronic and subacute severe and moderate groups combined, probably severe more than moderate. A reason for thinking this is that Schmorl's "florid" rickets was apparently unattended by healing in the form of inorganic salt deposition. But, as already pointed out, in our experience, the discovery of either subacute or chronic rickets without scattered foci of inorganic deposition either in the proliferative cartilage or in a metaphysis was a great rarity, although we did find cases of acute and subacute severe rickets in which fresh depositions of calcium-phosphate everywhere were lacking. We were completely unable to make a satisfactory separation of "healing"

rickets although in some of our cases obviously fresh healing was certain since deposits of blue staining material were present in the provisional zone of the proliferative cartilage; in many other cases there was evidence that the healing had been temporary and the rickets had started up again. In other words our healing group was too subject to error in interpretation to be included as an entity. If we had classified healed rickets as a separate group our cases would have been scattered throughout the series.

Schmorl's data came out exactly as the clinician expects from his knowledge of rickets, namely that "beginning" rickets would be most commonly encountered in the early months, that chronic and subacute severe rickets would predominate in the middle period and that toward the end of the second year healing and healed rickets would be found with increasing frequency.

The prevalence and severity of rickets in our series was greater in the negro than in the white subjects. This observation conforms to what was encountered in the older age group already reported (1) and, of course, has been the general experience clinically.

In the older child there is as might have been expected, a difference in the seasonal prevalence of rickets (1); more cases occur in the winter months than in any other. Such a variation has not found in the present study among children dying during the second year. Whether such an absence of seasonal variation is related to less contact with the outside environment can only be conjectural.

SUMMARY

The prevalence of rickets during each month of the first year and for the entire second year has been presented for a group of 1303 children whose skeletal tissues have been studied microscopically at autopsy.

Rickets was more prevalent and severe in the negro than in the white child.

Prematurity has no effect on the prevalence of rickets in this series of subjects.

In Baltimore season does not appear to play a rôle in the prevalence of rickets among this age group.

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PLATES

PLATE I

FIG. 1. Rickets, acute, slight. Arrows indicate several areas in cartilagenous matrix between hypertrophic cells when deposition of inorganic materials is lacking (as indicated by failure to stain with hematoxylin).

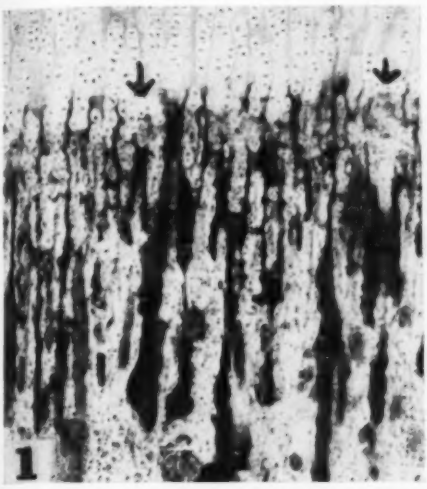
FIG. 2. Rickets, acute, slight. Higher power to show area in matrix between several rows of hypertrophic cells where deposition of inorganic materials is faulty.

FIG. 3. Rickets, acute, slight. Focus of defective calcification in cartilage.

FIG. 4. Rickets, acute, moderate. Large focus of defective calcification in cartilage.

FIG. 5. Rickets, acute, severe. Except for a few areas there is virtual cessation of lime salt deposition in provisional zone of calcification.

FIG. 6. Rickets, acute, severe. Sudden and complete cessation of deposition of inorganic materials in areas of hypertrophic cells. The suddenness is evidenced by the adequacy (dark staining material) beneath.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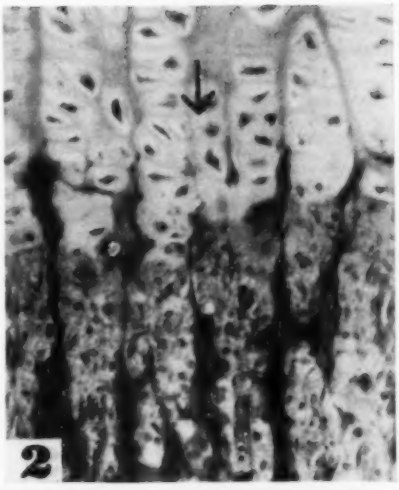
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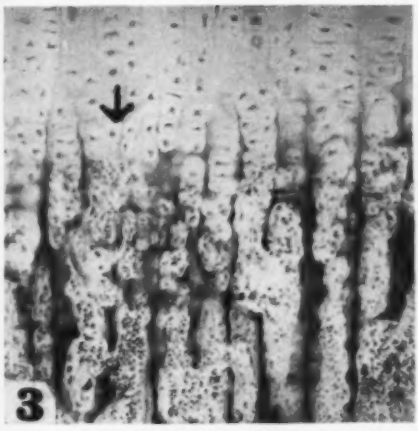
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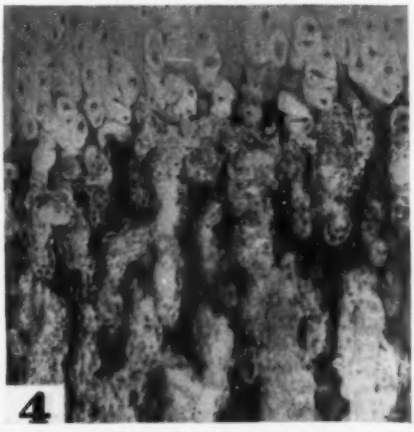
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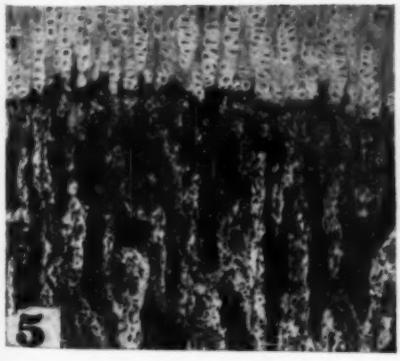
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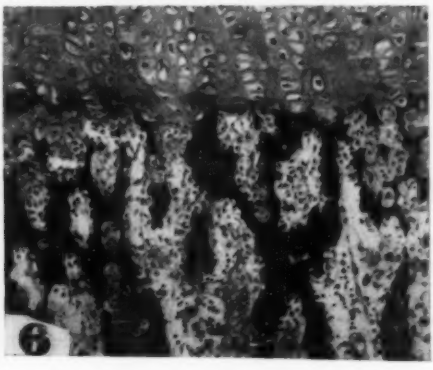
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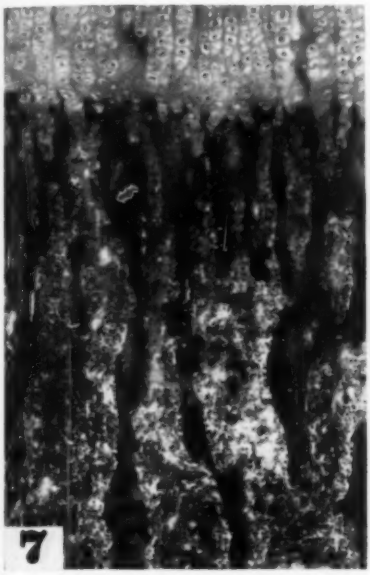
PLATE II

FIG. 7. Rickets, acute, severe. Marked defects in calcification in provisional zone with apparent adequacy up to very recently.

FIG. 8. Rickets, acute, severe. High power to show extensive area of defective lime salt deposition in cartilage.

FIGS. 9 and 10. Rickets, subacute, moderate. Low and high power of same preparation to show extensive defects in calcification as well as beginning irregularity in invasion of cartilage by blood vessels.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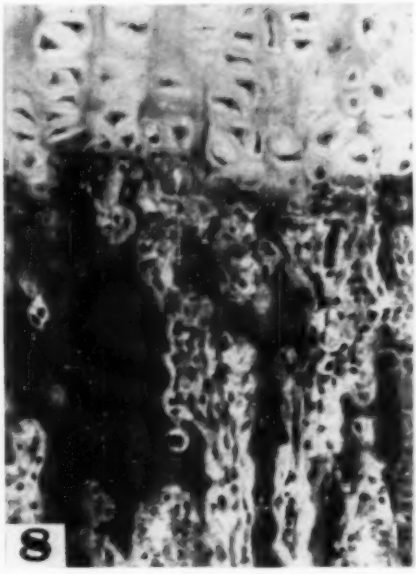
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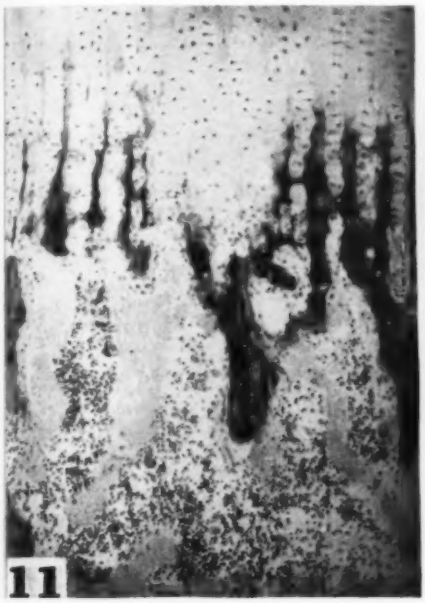
PLATE III

FIG. 11. Rickets, subacute, slight. There is a large focus of faulty calcification with increase in width of hypertrophic cell zone.

FIG. 12. Rickets, chronic, moderate. Defect calcification and marked irregularity in invasion of cartilage are prominent.

FIGS. 13 and 14. Rickets, chronic, severe. Sections of rib (13) and upper femur (14) from same individual to show marked difference in degree of involvement due to variation in rate of growth. (Same magnification.)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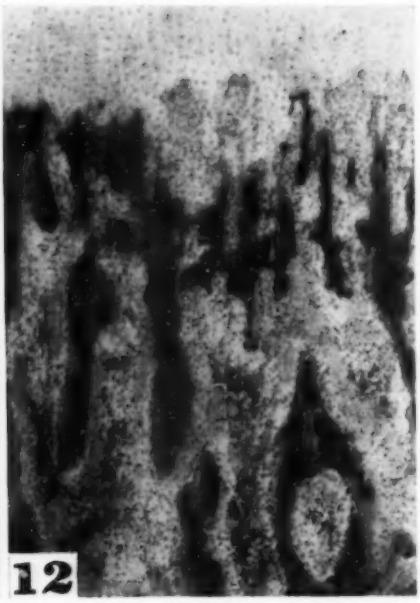
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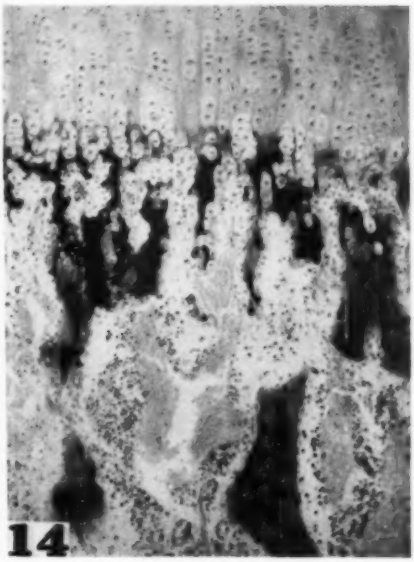
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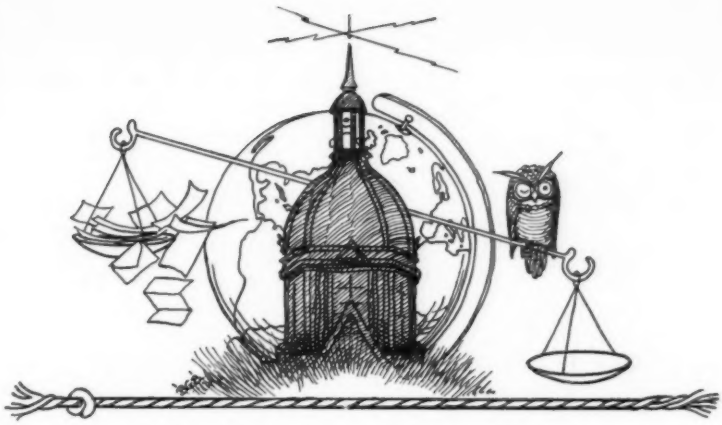

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Lehrbuch der Inneren Medizin. Edited by HELMUT DENNIG. Vol. I, 972 pp., Vol. II, 1060 pp., each volume Ganzl. DM 41. *Georg Thieme Verlag, Stuttgart, Germany.*

Lehrbuch der Inneren Medizin, edited by Dr. Helmet Dennig of Stuttgart, is an excellent textbook written by a group of ten German physicians. The chapters are almost all clearly written in a style which the amateur reader of German will not find too imposing. The articles are surprisingly up to date, as if there were relatively little lag between the time that they were written and the time of publication. For example, there is an extensive discussion of the Coxsackie viruses. Needless to say, the emphasis of the authors is frequently different from that of American writers. Neosalvarsan is still recommended for the treatment of syphilitic aortitis. There is a long account of the effects of starvation. The article on periarteritis nodosum is brief, and prolonged searching did not reveal any section on lupus erythematosus disseminata, perhaps a commentary upon your reviewer's command of German. The bibliographies at the end of each section are brief and include only monographs and what are usually secondary sources. The format in which this book has been published is attractive, as are the numerous illustrations. However, it is disconcerting to find that each of the two volumes is indexed separately.

Despite its excellence, there seems little reason why the American reader would prefer this book to the domestic variety. No doubt, however, in the words of Dr. Frank Sullivan Arbuthnot, it should be on the desk of every German physician.

OSCAR D. RATNOFF

Signs and Symptoms: Applied Pathologic Physiology and Clinical Interpretation, 2nd edition. Edited by CYRIL MITCHELL MACBRYDE. 783 pp., $10.00. *J. B. Lippincott Co., Philadelphia, Pa.*

This important book is now in its second eidtion. In it the editor and the 25 other contributors present physiological explanations for a variety of important symptoms and signs manifested by patients. Emphasis is laid particularly on the fact that in many illnesses the

correctness of diagnosis depends predominantly upon an accurate elucidation of the symptoms of illness during the course of taking a patient's history.

The volume presents discussions of 30 different symptoms, each in chapter form with an individual bibliography; included are headache, other types of pain, neurological, pulmonary, cardiovascular and gastro-intestinal symptoms, disturbances of consciousness, skeletal symptoms, nutritional and general metabolic symptoms, bleeding, jaundice, pigmentation, and itching. There is a brief chapter on nervousness and fatigue. Dr. MacBryde has selected the associate authors from medical centers throughout the country. The book is legibly printed and there are abundant diagrams and other illustrations. A few typographical errors are noted. The techniques employed in arranging the bibliographies vary from chapter to chapter. An occasional diagram is inadequately titled.

The volume contains much information found in standard medical texts. However, a distinctive feature of its compilation is that it presents to the reader in consecutive and orderly form the principal causes for the *symptoms* that in many diagnostic studies are major clues to correct diagnosis. Concomitantly, physiological information is presented that gives the reader an increased understanding of the mechanisms of disease processes. The reader of a standard textbook of medicine, which presents in individual sections the complete picture of single diseases, must frequently consult its index to obtain information on the various causes of a given symptom. Even if the index is compiled adequately enough to permit its use in this respect, many physicians find it inconvenient to refer to the several sections of a standard text in which the information they require for the interpretation of a symptom may be distributed. Hence, in this respect the volume under discussion is a useful supplement to other texts on medicine.

This well-conceived and up-to-date volume by Dr. MacBryde and his co-contributors is recommended to medical students, who, with other physicians, will find the chapter bibliographies helpful adjuncts. It can also be recommended to teachers and practitioners of medicine as a very useful source of information on the various causes of a given symptom and on the disturbances in physiology resulting from disease.

PALMER H. FUTCHER

The Story of the Adaptation Syndrome. By HANS SELYE. 225 pp., $4.50. *Acta, Inc., Montreal, Quebec, Canada.*

The embattled reviewer is finally aware that he could leave this odd and attractive little book on his desk for another six months or for six years without any definitive statement forthcoming. Embracing, as it does, the conclusions of twenty years' research and polemic in a field of sands still shifting vigorously with every monthly wind of publications, it would be unfair and unwise to delimit Selye either as hero or villain. The volume, rather, calls for comment first as regards its form and then in connection with a few trial balances which may reasonably be made regarding the research.

The type of book is something of a rarity—a scientific work in the *tu* person. It grew out of a series of lectures to medical students and doctors; it is part autobiography, part crusade, and part Adaptation Syndrome. Selye and reader rapidly become friends. We see the cramped wooden cubicles and feel the cold blasts of the Montreal winters blowing across the McGill Medical Building in 1935—stressing, as it were, the Stressor. We see this dissolve into the great Institute of Experimental Medicine, with the not unexpected comment that those early rugged years were the best. We are allowed to share the author's gentle gibes at himself for taking two years before he thought to incriminate the pituitary as the activator of the adrenal in the stress mechanism; in consolation, we share his triumph over the benighted professor who chastised him in the early years for studying the "pharmacology of

dirt." In the same vein, we are brought up to the present and his recent work, involving growth hormone (called here, with Selye's unfortunate habit of neologism, STH for somatotrophic hormone) somewhat as an antagonist to the ACTH-cortisone type of physiological activity.

Selye is free of rigid doctrine—his critics have found him too flexible rather than stubbornly adherent to his complex theories. He helpfully lists ten main objections that have been raised against the stress concept, and after answering these with admittedly varied measure of success, goes on himself to suggest anticipated objections—"A few additional doubtful points that have not yet been hurled against me."

No one will deny that Selye has influenced 20th Century physiology. But, in back of the point and counterpoint of theory and rival interpretation lies the experimental basis for the General Adaptation Syndrome as it now stands. It may be worthwhile to indicate several places where laboratory evidence clearly stands out for judgment. On the positive side, and by no means a complete tally: 1) The lesions produced in rats by steroids lacking an 11-oxygen moiety are certainly reproducible. 2) Similarly, the bacteremia associated with cortisone administration has been observed in many laboratories. 3) Although Selye disclaims credit for leading to the Mayo discovery of ACTH and cortisone for the rheumatoid diseases, he was able to conclude from his experimental work in 1944 "that the adrenal cortex may play an important role in the pathogenesis of rheumatic and rheumatoid disease in man." Against Selye it must be said that he has only worked with the rat, and at least certain important effects (cf. cold stress) cannot be reproduced in other species. Perhaps relevant to this is the question of whether the rat lesions are equivalent to those found in the "collagen" diseases of man. But most vulnerable of all to criticism are theories and even facts that involve the pituitary hormones, particularly corticotropin and growth hormone. For Selye treats these as though they were pure substances, and poises them delicately in his system of injury, adaptation and defense, usually in an antagonistic relation but often in synergism. But even the best samples of these two hormones are grossly impure (containing, for example, even pressor-antidiuretic and melanophore activity), and moreover, are mutually contaminated with each other! Until some of these chemical questions are resolved, the physiological speculations will be dangerous and almost certainly misleading.

Endocrinologists are moving away from the "adrenocentric position" demanded by Selye, and have come to the view that stress acts peripherally and is only modified by the adrenal cortex. This does not necessarily detract from the great contribution of Selye's in provoking emphasis on stress or, indeed, on the adrenal. From the tone of this volume it seems evident that he will always be ready to change views. Or more appropriately, adapt them. This will unquestionably be necessary as biochemical work on stress catches up to the pathological, and as the pituitary hormones become purified.

Meanwhile, those who prize the careful and "tight" approach to research will continue to ignore or to attack Selye; he will go his own way believing that "our facts must be correct ... theories need not be." It is hard to think that the world is too small to contain both sorts of worker, and there is abundant evidence here and elsewhere that the future often belongs to the biological sport.

THOMAS H. MAREN

Annual Review of Medicine. WINDSOR C. CUTTING, Editor. HENRY W. NEWMAN, Associate Editor. Editorial Committee: J. S. L. BROWNE, A. M. BUTLER, K. S. GRIMSON, E. M. MACKAY AND S. C. MADDEN. 442 pp., $6.00. *Annual Reviews, Inc. Stanford, California.*

"Annual Reviews" are now so well known in so many of the medical and biological sciences that one may easily predict the types of review to be found in this third volume of

Annual Review of Medicine. There is the expected mixture of good and bad. Among the reviews which excel, because of their clairty of presentation, are; "Infectious Diseases" by L. Thomas; "Diseases of the Urinary System" by J. Leutscher; and "Diseases of the Reproductive System" by J. McArthur. Thomas' review is particularly timely since he personally studied "The Effects of Cortisone and ACTH on Infection."

One may, however, wonder why this book is not called an Annual Review of American Medicine. With one outstanding exception (Nutrition) none of the reviews makes a real attempt to follow what has happened elsewhere in the world. J. N. P. Davies, who writes from the Department of Pathology, Makerere College Medical School, Uganda, East Africa, has performed a great service in his review "Nutrition and Nutritional Diseases." His beautifully written thirty page story of nutrition in the tropics should be read by medical and public health workers the world over. Much of it is concerned with "Kwashiorkor" a nutritional deficiency of African children. Study of this syndrome has interest for all of medicine, because of the profound effect on the liver and pancreas and the likelihood that explanation of this effect will help us understand many obscure relationships between hepatic and pancreatic function. Its prevalence among the children of Africa is a challenge to this "one world."

This reviewer of the reviews hopes that the editors will do more to keep us up to date about medicine elsewhere. Knowledge of medicine in England, France and Scandinavia is not identical with that covered by some 2500 references, chiefly from this country.

FREDERIK B. BANG

Problems of Aging. Transactions of the Fourteenth Conference, September 7–8, 1951. Edited by NATHAN W. SHOCK. 138 pp., $3.00. *Josiah Macy, Jr. Foundation, New York.*

At the fourteenth Macy conference on problems of aging, held in St. Louis shortly before the Second International Congress on Gerontology, there were participating, in addition to the regular panel members, guests from England, Denmark, and Switzerland. Discussion centered upon means of bringing together available knowledge in the broad fields of medicine sociology, economics, and medical services as they apply to gerontology. It is obvious from this list of topics that talk entered fields where exact knowledge is hard to come by and where it is even difficult to know how to go about getting the facts. Nevertheless, it is fruitless to deny that the problems of geriatric medicine extend beyond the ordinary confines of medical practice, and the record of this conference will be of particular interest to those who wish an introduction to several types of sociological thinking.

Those concerned with the problem of rehabilitation and with the problem of the most economical use of hospital beds will find that much spade work has been done by the gerontologists, and a good deal of the experience of gerontologists along these lines would appear applicable to general hospital practice.

It is, of course, not possible to criticize the content of this report, much of which is intended as speculation. It is evident that the serious efforts of the conferees did not really solve anything, nor were they intended to, but they occasionally bogged down in semantics and they ran headlong into that old bugaboo problem of defining "adequate" medical care. The difficulties of large scale planning are so enormous, and the basis for it so tenuous, that the most attractive immediate approach would seem to lie in examination of modest experimental units, some of which already exist.

KENNETH L. ZIERLER

Spatial Vectorcardiography. (American Monograph Series). By ARTHUR GRISHMAN AND LEONARD SCHERLIS. 217 pp., $6.00. *W. B. Saunders Co., Philadelphia, Pa.*

Spatial vectorcardiograms from approximately 400 subjects form the background of this monograph, in which the authors wish to "present those features of spatial vectorcardiog-

raphy which make it a valuable adjuvant to clinical electrocardiography." The abundant illustrations include frontal, sagittal, and horizontal vectorcardiograms and mutliple lead electrocardiograms from about 140 of these cases. These records are the most valuable feature of the book, giving the reader an opportunity to confirm or revise the conclusions in the text for himself. The authors' "cubical" system of electrode placement was used in all cases, and a brief discussion of other lead systems is included. The normal vectorcardiogram is described, as well as the changes which appear in various abnormal situations, principally ventricular hypertrophy, bundle branch block, and myocardial infarction.

Considerable attention is given to the relation between spatial vectorcardiograms and the conventional extremity and precordial electrocardiograms. The authors, together with a number of other workers in this field, believe abnormal complexes in these leads can be interpreted rationally in terms of abnormalities of the vector loop. They point out, for example, that in myocardial infarction the various ECG patterns produced by infarcts in different locations can be readily understood in terms of a single vectorial abnormality, namely the subtraction of electromotive force previously contributed by the infarcted muscle.

A second important aim of recent vectorcardiographic research has been the search for new diagnostic criteria in the spatial vectorcardiogram which might be absent or inaccessible in the usual ECG leads. In this category, the authors describe a method for differentiating between ventricular hypertrophy and bundle branch block by the speed and direction of rotation of the loops. Unfortunately, they define bundle branch block solely in terms of a vectorcardiographic pattern, and since they reject any definition of bundle branch block based on duration of the QRS, and anatomical definition is lacking, the validity of this point remains open to question.

WILLIAM R. MILNOR

Correlative Cardiology: An Integration of Cardiac Function and the Management of Cardiac Disease. By CARL F. SHAFFER AND DON W. CHAPMAN. 525 pp., $9.50. *W. B. Saunders Co., Philadelphia, Pa.*

The student of cardiology, whether he is a medical graduate or still in medical school feels a desire for a treatise on the subject which unlike the standard texts would summarize and correlate all knowledge about the heart beginning with anatomy and embryology and proceeding through physiology and pharmacology to clinical considerations. Shaffer and Chapman intend their book to fill this desire. The reviewer approached it with pleasant anticipation which was increased on preliminary survey of its format; it is not a synopsis but rather a full-length text which organizes the material in outline fashion giving the student both the skeleton and the meat of the subject. The bibliographies are short and carefully selected from recent and readily available material. With appropriate marginations by the student himself the book should be an unusually useful one.

On close perusal, however, faults in the book come to light which make it not only of questionable usefulness but also seriously confusing to the student for whom it is intended.

A principal flaw is the careless and inaccurate labelling of figures. The location of the characteristic machinery murmur of patent ductus is indicated as the *right* second interspace (P. 281); relative cardiac dullness is labelled absolute cardiac dullness and vice versa (P. 67); the arrows indicating direction of movement of the ventricular border in systole and with paradoxical movement as in aneurysm are pointing 180 degrees the wrong way (P. 131); a diagram of the heart in complete dextrocardia is labelled partial dextrocardia (P. 268); and so on (P. 18). Another fault of the illustrations is the inclusion of meaningless charts such as that on the "relationship of digitalis and glycosides" on P. 240—meaningless because of lack of explanation in the text.

In the second place inaccurate statements appear here and there: "The velocity of the pulse is dependent upon the diastolic pressure" (P. 32). "The increase in force in systolic

contraction (from digitalis) automatically increases the refractory period" (P. 238). On P. 55 the misconception appears to be perpetuated that cardiac hoarseness is due to pressure of the left auricle on the left recurrent laryngeal nerve—an anatomical impossibility. The reviewer is dubious that 50% of pheochromocytomas are palpable as abdominal tumors (P. 400), that basilar diastolic murmurs are heard 2 or 3 times more commonly than apical diastolic murmurs in severe anemia (P. 380), that primary neoplasms of the heart are more common than metastatic involvement (P. 387), that the oral digitalizing dose of digitoxin may be as high as 5.0 mgms. (P. 239), that the ischemic zone surrounding an infarct is usually more extensive in the subepicardial region (P. 178). Poor paragraph construction, as in paragraph A 3, P. 257, is disturbing to the reviewer and confusing to the student.

On the whole the book is not very inspired or inspiring beyond its general organization. For example the section on electrocardiography, although adequate, yet is on the whole a mediocre presentation. This reviewer for the reasons stated hesitates to recommend it to the student, for whom it was expressly intended, and seriously doubts that, in its present form, it is a worthwhile addition to the excellent presently existing textbooks of cardiology.

VICTOR A. MCKUSICK

Manual of Electrocardiography. By BENJAMIN F. SMITH. 215 pp., $4.50. *Elsevier Press, Houston, Texas.*

The opening chapters of this book are devoted to instruments, cardiac anatomy, electrical theory, and electrocardiographic leads and nomenclature. Eighteen pages are devoted to a description of "the electrocardiographic position of the heart" with X-rays and electrocardiograms; but little indication is given concerning the usefulness of such information. There are chapters on disturbances of the heartbeat and of conduction, on normal ventricular potentials, and on cardiac hypertrophy, acute cor pulmonale, myocardial infarction, and pericarditis. Fifty pages are occupied by a section on "correlation of electrocardiographic and autopsy findings," in which autopsy reports and uninterpreted electrocardiograms are presented without comment. The patient's history is not given in any of these cases except for dates of the electrocardiograms and of the patient's death, the intervals between these two events ranging from less than one day to about 65 days. Whether the reader will profit from such a "correlation" is debatable, especially since the beginner probably will be unable to interpret the electrocardiograms. The final section, entitled "pen pictures of cardiology," contains miscellaneous clinical information concerning various cardiac conditions, and probably has no place in such a volume.

There have been so many books published on electrocardiography, that the reviewer should base his judgment in part upon how the particular volume fulfills the author's objectives in offering it to the public. This manual is "not intended to cover all aspects of the subject; the real purpose is . . . to *start* the student on the road to becoming an electrocardiographer, not a mere pattern recognizer." Viewed in this light, one must state that this volume does not give the reader a start sufficiently good to warrant recommending this text for that purpose.

SIDNEY SCHERLIS

Primary Carcinoma of the Liver: A Study in Incidence, Clinical Manifestations, Pathology and Aetiology. By CHARLES BERMAN. With a Foreword by SIR ERNEST KENNAWAY and an Introduction by HENRY GLUCKMAN. 177 pp., 35s. net. *H. K. Lewis & Co. Ltd., London, England.*

This monograph is based on a detailed clinical and pathological study of 75 cases of primary cancer of the liver occurring in the South African Bantu (34 of the patients were in

the personal care of the author!) together with data on some 700 other cases from South African sources. The result is highly commendable. Berman's monograph collects together important material for the clinician, the pathologist, and the cancer researcher. The illustrations are excellent and numerous. The bibliography with its some 700 references is an indication of Berman's comprehensive coverage of the subject. His delineation of the following five clinical types would appear to have merit: Group 1. Frank liver cancer, i.e., cases with symptoms secondary to involvement of the liver. Group 2. Acute abdominal cancer, i.e., cases with a picture suggesting an acute abdominal accident. Group 3. Febrile cancer. Group 4. Occult cancer, i.e., cancer discovered incidentally. Group 5. Metastatic cancer, i.e., cases with symptoms or signs of metastases as the initial manifestation. He lists 62.7%, 8%, 8%, 16%, and 5.3%, respectively, for the relative incidence of these five types. In Baltimore, at least, primary liver cancer is by no means of infrequent occurrence—five or six new cases are recognized at the Johns Hopkins Hospital each year—and it frequently comes up for consideration in differential diagnosis. Berman's monograph is a welcome contribution.

VICTOR A. MCKUSICK

Textbook of General Surgery, 6th edition. By W. H. COLE AND R. ELMAN. 1154 pp., $12.50. *Appleton-Century-Crofts, Inc., New York.*

The sixth edition of this excellent textbook upholds and enhances the standards maintained since its initial appearance in 1936. Perhaps to be singled out for particular commendation are the sections on 1) Endocrinology, 2) the Antibiotics, 3) Postoperative Care, and 4) Fluid Balance and Nutrition, the last having been especially anticipated since Dr. Elman has done so much fundamental work in the subject.

Arrangement of the material is good since it is presented from a physiological viewpoint. The book will prove helpful not only to students but also as a reference book to physicians whose forte lies in fields other than surgery, for the style is exceptionally readable.

Physically the book is constructed sturdily with excellent weight binder's board, good paper, and the desirable use of headbands. The printing is of the finest caliber with the exception of a number of illustrations that have lost considerably in reproduction. The weight of the book is one factor that could be overcome by printing on a lighter weight stock.

With the one comment that the more common surgical diseases might have received more discussion without concern about over emphasizing surgical technics, the book is heartily recommended to its old friends as being thoroughly up-to-date, and to its to-be new friends as an excellent systematic survey of the field of surgery.

DAVID H. LYNN

BOOKS RECEIVED FOR REVIEW

Brain Surgeon. The Autobiography of Dr. William Sharpe. Foreword by JOHN HAYNES HOLMES. 271 pp., $3.75. *The Viking Press, Inc., New York.*

Carbohydrate Metabolism. A Symposium on the Clinical and Biochemical Aspects of Carbohydrate Utilization in Health and Disease. Edited by VICTOR A. NAJJAR. 134 pp., $4.00. *The Johns Hopkins Press, Baltimore, Md.*

Connective Tissues. Transactions of the Third Conference, February 14–15, 1952. Edited by CHARLES RAGAN. 166 pp., $3.50. *Josiah Macy, Jr. Foundation, New York.*

Employment Problems of Disabled Youth in Glasgow. Medical Research Council Memorandum No. 28. By T. FERGUSON, A. N. MACPHAIL AND MARGARET I. MCVEAN. 66 pp., 3s. net. *Her Majesty's Stationery Office, London, England.*

Gynecologic and Obstetric Pathology with Clinical and Endocrine Relations, 3rd edition. By EMIL NOVAK. 595 pp., $10.00. *W. B. Saunders Co., Philadelphia, Pa.*

A Method of Anatomy. Descriptive and Deductive, 5th edition. By J. C. BOILEAU GRANT. 870 pp., $7.00. *The Williams & Wilkins Co., Baltimore, Md.*

Nerve Impulse. Transactions of the Third Conference, March 3 and 4, 1952. Edited by H. HOUSTON MERRITT. 176 pp., $3.50. *Josiah Macy, Jr. Foundation, New York.*

Physiological Bases of Gynecology and Obstetrics. (American Lecture Series). By S. R. M. REYNOLDS. 156 pp., $5.50. *Charles C Thomas, Springfield, Illinois.*

Rypins' Medical Licensure Examinations. Topical Summaries and Questions, 7th edition. Edited by WALTER L. BIERRING. 856 pp., $8.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Viral and Rickettsial Infections of Man, 2nd edition. By THOMAS M. RIVERS. 719 pp. $7.50. *J. B. Lippincott Co., Philadelphia, Pa.*

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